Amines in Fish Muscle. V. Trimethylamine Oxide Estimation

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ABSTRACT

The Lintzel method of trimethylamine oxide determination was found to give low results when less than 2 to 5 mg. oxide nitrogen was present. By suitable modification this could be reduced to 2 mg. above which a constant 96 per cent recovery was obtained. However, many fish extracts contain only 0.5 to 2 mg. nitrogen in the aliquot which can be used for the reduction.

A modified Norris and Benoit procedure, which is much simpler to use, was developed. This gave about 96 per cent recovery down to about 0.4 mg. nitrogen. Extraction of the fish muscle with either trichloracetic acid or formaldehyde was satisfactory.

INTRODUCTION

In using the Lintzel method (Beatty, 1938) for the determination of trimethylamine oxide in fish extracts, very incomplete recovery was obtained with samples of low oxide content (Dyer *et al.*, 1946; Tarr, 1941). No quantitative comparison of the methods for the determination of trimethylamine oxide could be found in the literature.

Cook (1931) and Reay (1938) determined trimethylamine oxide in fish muscle by reduction with stannous chloride and hydrochloric acid, followed by estimation of the trimethylamine formed. Beatty (1938) found this method gave low recovery.

Lintzel (1934) determined the oxide in urine by reduction with Devarda's alloy in the presence of hydrochloric acid at 65°C. The trimethylamine formed was determined by aeration of the alkalinized mixture into standard acid, formaldehyde being added to bind any ammonia present. A modification of this method using distillation under reduced pressure was used by Beatty (1938, 1939).

Norris and Benoit (1945) again modified Lintzel's procedure by carrying out the reduction in normal hydrochloric acid using 0.5 g. Devarda's alloy per 5 ml. of test solution, and found reduction was complete in 15 minutes at 95°C. Choline, betaine, methylguanidine, creatine, aminoacids, and proteins did not interfere.

Jakobsen (1944) and Ronold and Jakobsen (1947) used titanous chloride as the reducing agent.

Using the above methods the recovery of trimethylamine oxide from both standard solutions and from fish muscle extracts has been investigated and a procedure giving satisfactory recovery at considerably lower concentrations has been developed.

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EXPERIMENTAL

Trimethylamine oxide, $(CH_3)_3NO.2H_2O$, prepared by the method of Dunstan and Goulding (1899) was used. The nitrogen content determined by the Kjeldahl method agreed with the theoretical value.

The results obtained by Lintzel's method as used by Beatty (1938) are shown in Figure I, Curve A. One to 5 ml. volumes of standard solutions of trimethylamine oxide containing 0.27 to 33.5 mg. nitrogen were used. Devarda's alloy, 0.5 g., was added to each sample followed by 2 ml. of 6 N hydrochloric acid every 10 minutes during an hour, the flasks being held at 65°C. in a water bath. The trimethylamine formed was determined by the colorimetric method

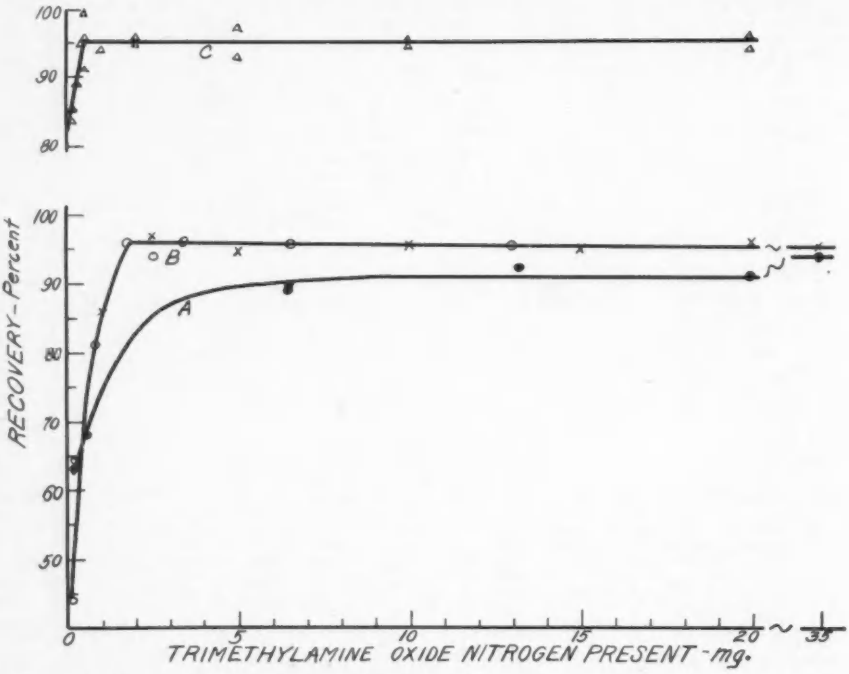

FIGURE 1. Recovery of trimethylamine oxide.
Curve A. Lintzel's method as used by Beatty.
Curve B. Modified Lintzel's method: O, pure trimethylamine oxide solutions; X, trichloracetic acid extracts of cod.
Curve C. Modified Norris and Benoit method.

of Dyer (1945) which was found to yield identical values with the distillation method used by Beatty and Gibbons (1937). The recovery decreases sharply when less than about 4 mg. trimethylamine oxide nitrogen is present in the reaction mixture, although the method is satisfactory at higher concentrations as used by Beatty (1939). Systematic study of the volume of the reaction

mixture, amount of Devarda's alloy and the amount of hydrochloric acid added showed that optimum reduction was obtained at 65°C, with an initial volume of 20 ml., addition of 1 g. Devarda's alloy, and 6 separate additions of 2.5 ml. 6 N hydrochloric acid at 10 minute intervals. A uniform recovery of 96 per cent with from 2 to 35 mg. trimethylamine oxide nitrogen present was obtained, Figure 1, Curve B, but below 2 mg. the recovery rapidly fell to below 50 per cent. Trichloracetic acid extracts of cod muscle gave the same curve when 96 per cent recovery was assumed at the higher concentrations, as shown in Figure 1, Curve B. Reduction was complete in about 10 to 20 minutes for pure trimethylamine oxide solutions but 50 to 60 minutes was required for the muscle extracts.

This low recovery at the lower concentrations is related to the trimethylamine formed, possibly due to binding of the amine by the alloy or to reduction to other compounds. When standard solutions of trimethylamine were carried through the reduction procedure, the recovery curve found was identical with that for the oxide.

Since in the proposed work it was often necessary to use aliquots containing only 0.5 to 2. mg. nitrogen, further investigation was undertaken.

Biological reduction of oxide added to peptone broth by growing cells of reducing bacteria was complete in less than three hours, but variable amounts of other amines were formed. Since it was found impossible to eliminate this extraneous amine formation which was equivalent to 10 to 30 per cent of the oxide present, this method was not satisfactory for quantitative use.

About this time the method of Norris and Benoit (1945) was published. This procedure was investigated in the same manner as above and a satisfactory modification developed. Results are shown in Figure 1, Curve C, showing 95 (± 3) per cent recovery of trimethylamine oxide nitrogen on both standard solutions and on fish extracts down to a concentration of about 0.4 mg. nitrogen in the aliquot used for the reduction. This was satisfactory for fish extracts, being equivalent to a lower range of about 16 mg. oxide nitrogen per 100 g. muscle, if 10 ml. of a 1 to 3 tissue extract were used.

Recommended Procedure

A suitable aliquot of tissue extract (see below) is made up to either 5 or 10 ml. with distilled water; then 1 g. of Devarda's alloy is added, followed by 2 ml. 6 N hydrochloric acid. The mixture is placed immediately in a boiling water bath for a period of 15 minutes. After cooling and making up to volume, usually 100 ml. but varying with the amount of trimethylamine present, an aliquot is taken for determination of trimethylamine by the colorimetric method (Dyer, 1945). In fish extracts the initial trimethylamine content is also determined and the oxide content calculated by difference.

Methods of Extraction

Beatty (1939) used either minced muscle or press juice extracts of fish muscle, Norris and Benoit (1945) used water extracts, and Ronold and Jakobsen (1947) worked with alcohol extracts. Trichloracetic acid extracts have

been used in most of the work in our laboratory (Dyer and Mounsey, 1945; Dyer, Dyer and Snow, 1946).

TABLE I. Trimethylamine oxide found, as mg. nitrogen per 100 g., in cod fillets by extraction with trichloracetic acid and formaldehyde and by direct distillation.

Direct distillation 2g.	Direct distillation 4g.	Trichloracetic acid extraction	Formaldehyde extraction
Cod fillet A			
		72	68
52	46	67	67
64	39	70	73
		65	65
		69	
		61	
Average 50		67.3	68.2
Cod fillet B			
69	64	77	67
70	65	71	80
		76	71
		73	73
		76	
		72	
Average 67		74	73

A comparison of the results obtained by extraction with trichloracetic acid, with formaldehyde, and directly on minced cod muscle was made (Table I). For trichloracetic acid extraction, 50 g. minced muscle was triturated with 50 ml. water and 100 ml. of 10 per cent trichloracetic acid, and let stand, usually overnight, but at least for two hours. For the formaldehyde extracts, 100 g. tissue was blended with 50 ml. formaldehyde (commercial, 40 per cent, diluted with an equal volume of water) and 150 ml. water in the Waring Blendor. Five and 10 ml. aliquots were taken for reduction, filtration being unnecessary. For direct determination on minced fish the Parnas method described by Beatty and Gibbons (1937) was used with 2 and 4 g. samples of the minced tissue.

There was no significant difference in the results obtained by using the trichloracetic acid and the formaldehyde extracts; both these extractants were also used satisfactorily in the survey of the trimethylamine oxide content of various fish species (Dyer, 1952). The values obtained by the direct distillation method on the minced tissue were low and variable. The use of trichloracetic acid or formaldehyde prevents spoilage with accompanying reduction of the oxide to trimethylamine, and the analysis need not be performed immediately, as would be necessary with water extracts. The direct determination of trimethylamine is also conveniently made on the same extracts.

REFERENCES

BEATTY, S. A. *J. Fish. Res. Bd. Can.*, **4**, 63-68, 1938.
Ibid., 229-232, 1939.

BEATTY, S. A., AND N. E. GIBBONS. *J. Biol. Bd. Can.*, **3**, 77-91, 1937.

COOK, A. S. *Can. Chem. and Met.*, **15**, 22, 1931.

DUNSTAN, W. R., AND E. GOULDING. *J. Chem. Soc.*, **75**, 1004-1011, 1899.

DYER, W. J. *J. Fish Res. Bd. Can.*, **6**, 351-358, 1945.
Ibid., **8**, 314-324, 1952.

DYER, W. J., AND Y. A. MOUNSEY. *J. Fish. Res. Bd. Can.*, **6**, 359-367, 1945.

DYER, W. J., F. E. DYER AND M. SNOW. *J. Fish. Res. Bd. Can.*, **6**, 403-413, 1946.

JAKOBSEN, F. *Tids. Kjemi Bergvesen Met.*, **3**, 14, 1944.

LINTZEL, W. *Biochem. Z.*, **273**, 243-261, 1934.

NORRIS, E. R., AND G. J. BENOIT. *J. Biol. Chem.*, **158**, 433-438, 1945.

REAY, G. A. *Rep. Food Inv. Bd. Gr. Brit.*, **1937**, 69-71, 1938.

RONOLD, O. A., AND F. JAKOBSEN. *J. Soc. Chem. Ind.*, **66**, 160-166, 1947.

TARR, H. L. A. *J. Fish. Res. Bd. Can.*, **5**, 211-216, 1941.

Amines in Fish Muscle. VI. Trimethylamine Oxide Content of Fish and Marine Invertebrates

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ABSTRACT

Original determinations of the trimethylamine oxide content of 60 species of fish are recorded, and 21 additional species have been studied by others. Elasmobranchs have the highest content of oxide, two to five per cent based on dry weight. Among teleost fishes, the amount increases from the lower to the higher orders, freshwater fish containing no oxide. Analyses of several species of marine invertebrates confirm earlier work showing that certain molluscs, echinoderms and other organisms contain trimethylamine oxide, often in quantities similar to those in the higher teleosts.

INTRODUCTION

One of the most distinctive characteristics of sea fish is its content of trimethylamine oxide. The earlier literature of a qualitative nature has been reviewed by Kutscher and Ackermann (1933). Quantitative studies of the trimethylamine oxide content of sea life have been made by Cook (1931), Beatty (1939), Reay (1939), Norris and Benoit (1945a) and Ronold and Jakobsen (1947); but these cover only limited parts of the sea fauna.

Because of the extensive use of the amount of trimethylamine formed from the oxide by bacterial action as a spoilage test on sea fish, particularly on cod, haddock, and similar fish (Dyer, 1945; Reay and Shewan, 1949) and on herring (Notevarp, Hjorth-Hansen and Bakken, 1944), the total trimethylamine oxide content becomes important. Recently it has been shown (Tarr, 1942; Ronold and Jakobsen, 1947) that the oxide is partially broken down during the canning of fish and that it influences corrosion and "maturation" in canned fish.

The data given in the present paper should be of aid to students of the comparative biochemistry of fish, but no attempt is made here to discuss this subject. The various fish species are grouped according to Pratt (1923) and Jordan and Everman (1896). Scientific and popular names given in the tables, are for the most part, those used in the American Fisheries Society's list (1948); however, when all our information is from another investigator, his names are used. Sometimes an alternative name commonly used in Canada has been included in brackets in the tables, and may also be used in the text.

The trimethylamine oxide content of many species is recorded here for the first time; the values for others are correlated with those found in the literature.

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EXPERIMENTAL

Fish were obtained, as available, from fish plants and shore boats; however, many of the rarer species were from fish brought to the laboratory for identification. They were either analysed immediately or rapidly frozen and stored before examination. In most cases analysis was made of a trichloracetic acid extract, 25 g. fish triturated with 75 ml. of 6.6 per cent trichloracetic acid, but sometimes formaldehyde extracts were used (Dyer, Dyer and Snow, 1952). Since the fish were usually dead when received, only the muscle was analysed. The trimethylamine oxide was determined in duplicate as described by Dyer, Dyer and Snow (1952). The trimethylamine content found in the various fish was almost always less than 1 mg. nitrogen per 100 g.; and was therefore neglected in the calculation of the oxide content. Moisture determinations (dried to constant weight at 105°C.) were made where possible so that the results could be calculated on a dry matter basis.

The results are shown in Tables I-III. The trimethylamine oxide contents found are expressed on the wet basis and on the dry basis where data were available. Moisture contents found are also given. The range and average of the values are given with the number of specimens analysed indicated in brackets. For purposes of comparison, the values given by Norris and Benoit (1945a), Beatty (1939), Cook (1931), Reay (1939), and Ronold and Jakobsen (1947) are included. Besides the fishes, certain members of the Mollusca, Echinodermata and Arthropoda are included since these are important food items for various fish.

The Arthropoda, particularly the copepods and decapods, which are very important fish foods, have been shown to contain appreciable amounts of trimethylamine oxide (Norris and Benoit, 1945a). The American lobster, *Homarus americanus*, was found to contain 125 mg. nitrogen per 100 g. as trimethylamine oxide (subsequent trimethylamine oxide contents in this paper will be expressed on this basis), whereas Reay (1939) found 122 in *Nephrops norvegicus*. Of the Mollusca, the Amphineura, Gastropoda and part of the Pelecypoda (namely, the clams, mussels and oysters), were found not to contain oxide (Table I). On the other hand, the cockles and scallops, and the Cephalopoda (the octopus and squid), contain quite large amounts.

The starfish (*Asterias vulgaris*) contains a small amount, about 20. The Atlantic seacucumber (*Cucumaria frondosa*) was found to contain about 80, whereas it was absent in the Pacific species (*C. miniata*) analysed by Norris and Benoit (1945a).

Since a large and important group of the marine invertebrates do contain significant amounts of trimethylamine oxide, the oxide does occur in the food of many fishes.

Proceeding to the fishes (Table II), the elasmobranchs have a very high oxide content, particularly the dogfishes and porbeagle in which about one per cent of the oxide is in the muscle tissues. On a dry weight basis this is about 4 to 5 per cent. The oxide content of skates varies from about 70 to 234 corresponding to 2 to 4 per cent on a dry weight basis. The Atlantic torpedo (*Tetranarce*

TABLE I. Trimethylamine oxide content of Mollusca and Echinodermata

Species	Trimethylamine oxide[a]	Author[b]
MOLLUSCA	*mg. N per 100 g.*	
AMPHINEURA		
Katharina tunicata—black chiton	0	N & B
Cryptochiton stelleri—giant chiton	0	N & B
GASTROPODA		
Anisodoris nobilis—sea slug	0	N & B
Thais lamellosa—snail	0	N & B
Littorina silchana—snail	0	N & B
Polinices heros—moonshell	0	D
PELECYPODA		
Mya arenaria—clam	0	D
Spissula solidissima—bar clam	0	D
Paphia straminea—clam	0	N & B
Saxidomus giganteus—clam	0	N & B
Macoma inquinata—clam	0	N & B
Mytilus edulis—mussel (Pacific)	0	N & B
Mytilus edulis—mussel (Atlantic)	0	D
Ostrea japonica—oyster (Pacific)	0	N & B
Ostrea virginica—oyster (Atlantic)	0	D
Cardium californiense—cockle	15, 18	N & B
Cardium corbis—cockle	32	N & B
Pecten hericius—scallop (muscle)	45–73(9)	N & B
(organs)	3–6(4)	N & B
Pecten hindsii—scallop (muscle)	42–110(7)	N & B
(organs)	7–12(3)	N & B
Pecten jordanii—scallop	45	N & B
Pecten grandis—scallop (Atlantic)	79–84(3)	D
CEPHALOPODA		
Polypus hongkongensis—octopus	24	N & B
Loligo opalescens—squid	150–156	N & B
Loligo pealeii—squid	110, 122	D
ECHINODERMATA		
ASTEROIDEA		
Asterias vulgaris—starfish	20	D
ECHINOIDEA		
Strongylocentrotus franciscanus—sea urchin	0	N & B
HOLOTHUROIDEA		
Cucumaria frondosa—sea cucumber	76–86	D
Cucumaria miniata—sea cucumber	0	N & B
Stichopus californicus—sea cucumber	0	N & B

[a]Number of samples is given in brackets.
[b]Authors listed are: N & B—Norris and Benoit (1945a); D—this paper.

occidentalis) and the ratfish (*Hydrolagus colliei*) contain a similarly high amount. This high oxide content coupled with a high urea content gives the Elasmobranchii a high osmotic pressure such that they are slightly hypertonic to seawater.

TABLE II. Trimethylamine oxide content of Marsipobranchii, Elasmobranchii and Holocephali.

Species	Moisture	Trimethylamine oxide		Author[a]
	Per cent	*mg. N/100 g. wet weight*	*% dry weight*	
MARSIPOBRANCHII				
Myxine glutinosa—hagfish	..	63	..	C
ELASMOBRANCHII				
Lamna nasus—porbeagle	78.5	200	4.9	D
Squalus acanthias—Atlantic spiny dogfish	75	190(5)	4.1	D
	..	267(6)	..	B
Squalus suckleyi—Pacific spiny dogfish	..	190	..	N & B
Squalus sp.	..	202, 267	..	R
Raja erinacea—little skate	76.4	86(2)	2.0	D
Raja scabrata—Atlantic prickly skate	81	37–160 = 73[b](5)	2.1	D
	..	51(2)	..	B
Raja senta—Smooth skate	..	99	..	B
Raja laevis—barndoor skate	..	234	..	B
Raja sp.—blue skate	..	205	..	R
Tetranarce occidentalis—Atlantic torpedo	83	116	3.7	D
HOLOCEPHALI				
Hydrolagus colliei—ratfish	..	180	..	N & B

[a]Authors listed are: C—Cook (1931); B—Beatty (1939); R—Reay (1939); N & B—Norris and Benoit (1945a); D—this paper.

[b]Average figures are given after the range; number of samples is in brackets.

The values for the teleosts vary considerably. The lower orders of teleosts (Orders M-Z in Table III) are low in oxide. The Clupeidae contain about 40 except for the Atlantic and Pacific herrings, which in our work average about 75. The Salmonidae are very low, below 25, as also are the smelt (*Osmerus mordax*) and the billfish (*Scomberesox saurus*). As found by earlier workers, there is little or no trimethylamine oxide in freshwater species. When freshwater fish spoiled, however, small amounts of trimethylamine were often found, confirming the results of Ronold and Jakobsen (1947). This probably arises from choline and similar compounds (Dyer and Wood, 1947) since no trimethylamine oxide can be detected in the fresh fish.

Of the Acanthopteri, the mackerel (*Scomber scombrus*), tuna (*Thunnus thynnus*) and the swordfish (*Xiphias gladius*) have quite low amounts. On the other hand, the rudderfish (*Seriola dumerili*), barrelfish (*Palinurichthys perciformis*), dollarfish (*Poronatus triacanthus*), striped bass (*Roccus saxatilis*) and blue perch (*Taeniotoca lateralis*) average about 60.

Most of the species of the Plectognathi are higher, averaging 76. Of these the family Gadidae are most important, and contain the largest amount of oxide of teleost fishes, averaging 88 for 6 species. Pollack (*Pollachius virens*) contains about 70; cod (*Gadus callarias*) 95; haddock (*Melanogrammus aeglefinus*) 70; hake (*Urophysis chuss*) 120; and cusk (*Brosme brosme*) 65. Cod thus contains about 2.7 per cent trimethylamine oxide calculated as per cent of dry weight, haddock about 1.9 per cent, and hake is almost as high as the dogfish, about 3.7 per cent. The Pleuronectidae are somewhat lower than the Gadidae, ranging from about 60 to 90 and averaging about 65.

In the teleosts there seems to be some evidence that the lower groups and particularly the anadromous fish contain less oxide than those in the higher groups. But there are many exceptions, and it should be interesting to study the comparative biochemistry of the trimethylamine oxide content of the divergent races in fish development.

No oxide was found in either the porpoise (*Phocaena phocaena*) or the blue whale (*Balaenoptera musculus*) Smith and Sharp (1946) also found no oxide in whale meat.

DISCUSSION

It is interesting to note the similarity in trimethylamine oxide content of the same or closely related species caught in different parts of the world. For instance in halibut, the Atlantic species (*Hippoglossus hippoglossus*) averaged an oxide content of 70, whereas the Pacific (*H. stenolepis*) contained 65. Atlantic cod (*Gadus callarias*) averaged 95, whereas a range of 67 to 75 was found by Reay (1939) in England, and a 50 to 92 by Ronold and Jakobsen (1947) in Norway. Similarly, haddock (*Melanogrammus aeglefinus*) averaged 70 in the present study, while Reay found 41 to 56 and Ronold and Jakobsen 47. Atlantic salmon (*Salmo salar*) averaged 24, while a Pacific salmon (*Oncorhynchus tshawytscha*) analysed 25; Norris and Benoit (1945a) found 10 to 12 in the latter. The Alantic herring (*Clupea harengus*) averaged 74 in our tests, whereas the results of Ronold and Jakobsen (1947) average only 36. The Pacific species (*C. pallasii*) contained 69.

In this connection it should be pointed out that when fish are stored in ice a considerable amount of leaching of the soluble constituents including trimethylamine oxide may occur (Dyer, Dyer and Snow, 1946), and therefore every effort was made in the present investigation to obtain fresh fish which had not been subjected to leaching in ice or copious washing.

Insufficient samples were obtained of most species to gain much information on the variation in age and size of fish. In large steak cod, approximately 90 cm. long, the trimethylamine oxide content averaged 107, with a range of 103 to 112 (8 samples). Medium cod, approximately 33 to 40 cm. in length, averaged 97, ranging from 80 to 115 (10 samples). Small cod, 20 cm. in length or less, averaged only 72, ranging from 67 to 77 (7 samples). Thus there is some evidence that the oxide content increases in the larger and older fish.

In yellowtails (*Limanda ferruginea*) likewise, the fish 40 to 43 cm. long averaged 78 ranging from 73 to 88, those 25 to 30 cm. in length averaged 56 with a range of 30 to 67. Those 23 cm. long averaged 34 ranging from 23 to 48. Only

TABLE III. Trimethylamine oxide in Teleostei.

Species	Moisture	Trimethylamine oxide		Author[a]
	per cent	*mg. N/100 g. wet weight*	*% dry weight*	
Order M. NEMATOGNATHI				
Ameiurus nebulosus—catfish	..	0(6)	..	B
Order N. PLECTOSPONDYLI				
Catostomus commersonnii—sucker	..	0(2)	..	B
Moxostoma aureolum—northern redhorse	82.3	0	0	D
Semotilus atromaculatus—creek chub	79.6	2(2)	..	D
Carassius auratus—goldfish	..	42	..	D
Order Q. APODES				
Anguilla bostoniensis—American eel	57.5	0(2)	..	D
	..	0(6)	..	B
Order S. ISOSPONDYLI				
Tarpon atlanticus—tarpon	83.6	43	1.4	D
Clupea harengus—Atlantic herring	72	67–81 = 74[b](7)	1.4	D
	..	42–110 = 90(8)	..	B
	..	10–64 = 36(116)	..	J
Clupea pallasii—Pacific herring	..	69	..	D
Clupea sprattus—herring	..	6–25 = 14(60)	..	J
Pomolobus chrysochloris—alewife	..	35	..	C
Pomolobus pseudoharengus—alewife (gaspereau)	79	33–52 = 44 (13)	1.1	D
		47(3)		B
Alosa sapidissima—American shad	68.5	45	0.77	D
Leucichthys artedi—shallow-water cisco	71.7	6	..	D
	..	0	..	C
Oncorhynchus tschawytscha—king salmon (spring salmon)	..	10–12	..	N & B
	..	25(2)	..	D
Oncorhynchus kisutch—silver salmon	..	9-12	..	N & B
Salmo salar—Atlantic salmon	70	24(2)	0.43	D
Salvelinus fontinalis—Eastern brook trout (sea-run)	76.4	13(2)	0.3	D
Osmerus mordax—American smelt	81	27(4)	0.1	D
Argentina silus—Argentine	78	122	3.0	D
Order V. HETEROMI				
Notacanthus nasus	..	133	..	D
Order X. HAPLOMI				
Esox lucius—pike	76.7	3	..	D
Fundulus heteroclitus—mummichog	..	14	..	D
Fundulus diaphanus—banded killifish	..	0	..	D
Order Y. SYNENTOGNATHI				
Scomberesox saurus—Atlantic saury (billfish)	72	17(2)	0.33	D
Order Z. HEMIBRANCHII				
Gasterosteus aculeatus—threespine stickleback	..	14	..	D
Order BB. ACANTHOPTERI				
Menidia notata—Atlantic silverside	82	28	..	D
Scomber scombrus—Atlantic mackerel	63	31–38 = 34 (3)	0.5	D
	..	41–54 = 48(10)	..	B

[a]Authors listed are: B—Beatty (1939); C—Cook (1931); J—Ronald and Jakobsen (1947); N & B—Norris and Benoit (1945a); D—this paper.

[b]Average figures are given after the range; number of samples is in brackets.

TABLE III—(*Cont'd*)

Species	Moisture	Trimethylamine oxide		Author[a]
	per cent	*mg. N/100 g. wet weight*	*% dry weight*	
Thunnus thynnus—bluefin tuna	67	4	..	D
Xiphias gladius—broadbill swordfish	69	31	0.5	D
	..	43	..	C
Seriola dumerili—amberjack (rudderfish)	76	60	1.4	D
Palinurichthys perciformis—black rudderfish (barrelfish)	80	59	1.6	D
Poronotus triacanthus—butterfish (dollarfish)	68	58	1.0	D
Perca flavescens—yellow perch	..	0(5)	..	D
Roccus saxatilis—striped bass (sea bass)	..	62	..	N & B
Morone americana—white perch	..	59	..	D
Taeniotoca lateralis—blue perch	..	67	..	N & B
Order CC. PLECTOGNATHII				
Sub-Order GYMNODONTES				
Mola mola—sunfish	86.5	70	2.8	D
Sebastes marinus—rosefish	80.5	92(2)	2.5	D
	..	67	..	R
	..	58	..	C
Sebastodes sp.—rockfish	..	22–67 = 53[b](5)	..	N & B
Sebastodes melanops—black rockfish	..	93	..	D
Sebastodes ruberrimus—red rockfish	..	90	..	D
Ophiodon elongatus—lingcod	..	84	..	D
Scorpaenichthys marmoratus—cabezone (bull cod)	..	65	..	N & B
Myoxocephalus groenlandicus—shorthorn sculpin	77	98	2.3	D
Myoxocephalus octodecemspinosus—longhorn sculpin	80	72–110 = 91 (4)	2.4	D
Hemitripterus americanus—sea raven	81	83–91 = 87 (4)	2.4	D
Cyclopterus lumpus—lumpfish	88	45	2.1	D
Suborder XENOPTERYGII				
Anarhicas latifrons—broadheaded catfish	79	56	1.5	D
Anarhicas lupus—Atlantic wolffish (common catfish)	84	53–68 = 63	2.1	D
Macrozoarces americanus—ellpout	80	69–73 = 71(2)	1.8	D
		59		C
Sub-order ANACANTHINI				
Merluccius bilinearis—silver hake	80	89–123 = 106(2)	2.9	D
Pollachius virens—pollack (pollock)	81	40–76 = 55(12)	2.5	D
	..	82–93 = 90(7)	..	B
Gadus morhua (callarias)—Atlantic cod	81	67–115 = 95(25)	2.7	D
	..	110–146 = 125(50)	..	B
	..	67–75	..	R
	..	50–92 = 66(7)	..	J
Melanogrammus aeglefinus—haddock	80	43–83 = 70(9)	1.9	D
	..	63–80 = 71(8)	..	B
	..	41–56	..	R
	..	30–58 = 47 (5)	..	J
Lota lota—burbot (freshwater ling)	..	22	..	C

[a]Authors listed are: B—Beatty (1939); C—Cook (1931); J—Ronald and Jakobsen (1947); N & B—Norris and Benoit (1945a); D—this paper.

[b]Average figures are given after the range; number of samples is in brackets.

TABLE III—(*Cont'd*)

Species	Moisture	Trimethylamine oxide		Author[a]
	per cent	*mg. N/100 g. wet weight*	*% dry weight*	
Urophycis chuss—squirrel hake	82.5	100–140 = 120[b](5)	3.7	D
	..	147–176 = 166(5)	..	B
Brosme brosme—cusk	80	65	1.8	D
Macrourus berglax—smooth spined rat-tail	83	85	2.7	D
Sub-order HETEROSOMATA				
Hippoglossus hippoglossus—Atlantic halibut	78	65–75 = 70(5)	1.7	D
Hippoglossus stenolepis—Pacific halibut	..	65	..	D
Hippoglossoides platessoides—American (Canadian) plaice	82	93	2.7	D
Limanda ferruginea—rusty dab (yellowtail)	81	21–88 = 62(10)	1.8	D
	..	78	..	B
Pseudopleuronectes americanus—winter flounder	82	64–29 = 70(30)	2.1	D
Glyptocephalus microcephalus—lemon sole	..	36	..	R
Glyptocephalus cynoglossus—witch	..	43–105 = 58(6)	..	B
Glyptocephalus sp.	..	27–29(3)	..	N & B
Order DD. PEDICULATI				
Lophius piscatorius—goosefish (monkfish)	83	42–75 = 59(2)	2.1	D

[a]Authors listed are: B—Beatty (1939); C—Cook (1931); J—Ronald and Jakobsen (1947); N & B—Norris and Benoit (1945a); D—this paper.

[b]Average figures are given after the range; number of samples is in brackets.

a total of 12 samples were analysed, however, but there is an indication of increase in the larger fish.

A considerable seasonal variation in the oxide content of herring was found by Ronold and Jakobsen (1947). However, no data on size or age of the fish sampled were given. In our work too few samples spread over the year were obtained but there was no indication of seasonal variation with herring, yellowtails, haddock or cod.

The metabolism and significance of the presence of trimethylamine oxide in fish is obscure. Several hypotheses have been advanced on its origin and purpose; but it should be stressed that our knowledge of biochemical processes in fish is very meagre and is drawn for the most part by analogy from mammalian metabolism.

1. Kutscher and Ackerman (1933) conclude that the trimethylamine oxide must arise by methylation and oxidation of the lower cleavage products of protein degradation, and that it plays a part in regulating the tissue osmotic pressure in seawater animals.

2. Possibly it may be formed as a detoxification endproduct of ammonia similar to urea, as a result of biological methylation. Baldwin (1947) points out that the water supply in marine teleosts may be insufficient to eliminate all the nitrogen in the form of ammonia. Thus the synthesis of the relatively innocuous trimethylamine oxide results in lessening the danger of ammonaemia. In elasmobranchs, the oxide is also produced, but in addition, they have evolved the still better mechanism of urea formation, and retain sufficient of both in the organism

to make them hypertonic to seawater. Thus the problem of water retention, ever present in sea fish, has been overcome by this group. The trimethylamine oxide contributes about 25 per cent of the osmotic pressure of the internal medium in elasmobranchs, about 40 and 35 per cent being contributed by the urea and salts respectively.

3. Kapeller-Adler and Vering (1931) and Reay (1939) have suggested that trimethylamine oxide originates in the food and is simply stored in the muscle. Benoit and Norris (1945) have indeed shown that young salmon grown on an oxide-free diet in seawater do not contain trimethylamine oxide, whereas on an oxide-containing diet their muscle oxide content rises to the normal level. It is shown by Norris and Benoit (1945a) and in the present paper that many marine invertebrates, especially decapods and copepods, which are a major item of food of many seafish, contain trimethylamine oxide in amounts comparable to seafish. However, although the food of most seafish undoubtedly does contain oxide, the suggestion that it is merely stored in the muscle does not seem reasonable in view of the relatively constant oxide level found in the tissue of each of the various species. There must be some system of regulation, at least in the elasmobranchs where it contributes to the osmotic pressure.

4. Trimethylamine can be produced by bacteria, similar to those of the digestive tract, from choline (Dyer and Wood, 1947). Norris and Benoit (1945b) have shown that oxide fed orally is rapidly eliminated in the urine of the rat, and Tarr (1941) has shown that both trimethylamine and the oxide appeared as the oxide in the urine of man. Grollman (1929) found a considerable amount of oxide in the urine of the monkfish and the results of Smith (1936) suggest the same for other teleosts. Thus some of the trimethylamine oxide in fish could originate from choline or similar compounds.

Smith (1931a, 1931b, 1932) has investigated the osmotic pressure relationships in teleosts and elasmobranchs. This subject is well reviewed by Florkin and Morgulis (1949), and by Baldwin (1947). Smith (1936) concludes that in the elasmobranchs while urea is secreted by the kidney it is later resorbed from the urine by special absorbing loops. The volume of the urine and the degree of resorption act as an osmotic pressure regulator. It seems possible that the trimethylamine oxide level may be regulated in the same way. Smith (1929, 1930) shows that ammonia and salts are excreated in large part through the gills, and some of his experiments indicate that trimethylamine oxide is partially excreted in the same manner (Baldwin, 1947).

Smith (1929) and Florkin and Morgulis (1949) show that the nitrogen excretion of marine teleosts consists chiefly of ammonia and trimethylamine oxide which are excreted mainly through the gills. A small part, of course, is eliminated through the urine (which is very small in amount in the marine teleosts). It would seem, then, that the trimethylamine oxide level must be regulated by the gill excretion. In freshwater fish, on the other hand, even if trimethylamine oxide were formed or was present in the food (which is doubtful), the copious urine excreted would lead to rapid elimination of any oxide present.

The mechanism of regulation of the oxide concentration of the tissues probably differs in the elasmobranchs and in the teleosts, because of the fundamental difference in urine excretion, and because the blood of the latter has been found to contain little or no oxide. In the elasmobranch *Squalus suckleyi* Norris and Benoit (1945a) found that the concentration in the blood was about one half of that present in the muscle. But in teleosts, no oxide was found in the blood of Pleuronectidae sp., *Sebastodes* sp. or in *Scorpaenichthys marmoratus.* In the present work, seven live pollack, *Pollachius virens,* were examined and in all specimens less than 0.5 mg. trimethylamine oxide nitrogen per 100 ml. was present in the blood. Thus there is a gradient between the tissue cells which contain 0.56 per cent trimethylamine oxide (wet basis) and the blood which contains less than 0.004 per cent. Since trimethylamine oxide seems to be concentrated in the tissue at relatively constant level, how is this gradient maintained? Perhaps the oxide is bound in an undiffusable combination in the living cells similar to that in which urea is assumed to exist in the tissue (Florkin and Morgulis, 1949). Certainly, the oxide is very diffusable in the dead muscle tissue (Dyer, Dyer and Snow, 1946). If the trimethylamine oxide in the teleost flesh were osmotically active, it would raise the osmotic pressure of the tissue and decrease the loss of water and the entrance of salt from seawater through the skin. Since the oxide is excreted via the gills, it must be carried by the blood, if only in very low concentration similar to that of ammonia.

The question of the origin of trimethylamine oxide in marine fish is thus only partially answered. Certainly some is present in the food of most, if not all, seafish. Part may arise from choline through bacterial agencies, and it may be synthesized from ammonia. The latter mechanism may be a genetic variation which is useful in some species as an ammonia detoxification process and serves at least in the elasmobranchs in maintaining osmotic pressure. We must still speculate, however, on its origin in the marine invertebrates.

SUMMARY

The trimethylamine oxide contents of the muscle of 9 species of marine invertebrates and 60 species of fish have been determined and compared with values given in the literature.

Many of the marine invertebrates contain oxide in amounts similar to teleost fish, showing that the food of many fishes is rich in oxide.

Elasmobranchs have the highest trimethylamine oxide content, from 75 to 250 mg. oxide nitrogen per 100 g. of tissue, or about 2 to 5 per cent of the dry weight.

The lower orders of the teleosts, including the Nematognathi, Plectospondyli, Apodes, Isospondyli, Haplomi, Synentognathi and Hemibranchii, contain either no oxide or else only small amounts, with the exception of the herring. The freshwater species were found to contain no oxide.

The Acanthopteri varied in trimethylamine oxide nitrogen content from 30 to 60 mg. per 100 g. tissue.

The Plectognathi, containing the important food fishes of the cod and flounder families, were all quite high in oxide, averaging between 60 and 120.

The same or closely related species of fish usually have very similar oxide contents regardless of the part of the world in which they are caught. There is some evidence that larger and older fish have higher oxide contents.

No trimethylamine oxide was found in the blood of pollack, *Pollachius virens*. It is suggested that the oxide originates partly in the food and partly as an ammonia detoxification endproduct. It is eliminated mostly via the gills, and therefore must be transported by the blood.

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REFERENCES

American Fisheries Society. *Trans. Am. Fish. Soc.*, **75** (1945), 355-397, 1948.

Baldwin, E. Dynamic aspects of biochemistry. 457 pp. Cambridge University Press, 1947.

Beatty, S. A. *J. Fish. Res. Bd. Can.*, **4**, 229-232, 1939.

Benoit, G. J., and E. R. Norris. *J. Biol. Chem.*, **158**, 439-442, 1945.

Cook, A. S. *Can. Chem. and Met.*, **15**, 22, 1931.

Dyer, F. E., and A. J. Wood. *J. Fish. Res. Bd. Can.*, **7**, 17-21, 1947.

Dyer, W. J. *J. Fish. Res. Bd. Can.*, **6**, 351-358, 1945.

Dyer, W. J., F. E. Dyer, and J. M. Snow. *J. Fish. Res. Bd. Can.*, **6**, 403-413, 1946.
Ibid., **8**, 309-313, 1952.

Florkin, M., and S. Morgulis. Biochemical evolution. 157 pp. New York: Academic Press, 1949.

Grollman, A. *J. Biol. Chem.*, **81**, 267-278, 1929.

Jordan, D. S., and B. W. Everman. *U.S. Nat. Museum Bull.*, No. 47. 3313 pp., 1896.

Kapeller-Adler, R., and F. Vering. *Biochem. Z.*, **243**, 292-309, 1931.

Kutscher, F., and D. Ackermann. *Ann. Rev. Biochem.*, **2**, 355-376, 1933.

Norris, E. R., and G. J. Benoit. *J. Biol. Chem.*, **158**, 433-438, 1945a.
Ibid., 443-448, 1945b.

Notevarp, O., S. Hjorth-Hansen, and K. Bakken. *Tids. Kjemi. Bergvesen Met.*, **7**, 59-61, 1944.

Pratt, H. S. Vertebrate animals of the U.S. 422 pp. Philadelphia: Blakiston, 1923.

Reay, G. A. *Rep. Food Inv. Bd. Gr. Brit.*, **1938**, 87-89, 1939.

Reay, G. A., and J. M. Shewan. *Adv. in Food Res.*, **2**, 343-398, 1949.

Ronold, O. A., and F. Jakobsen. *J. Soc. Chem. Ind.*, **66**, 160-166, 1947.

Smith, E. C. B., and J. G. Sharp. *Food Manuf.*, **21**, 371-377, 1946.

Smith, H. W. *J. Biol. Chem.*, **81**, 727-742, 1929.
Amer. J. Physiol., **93**, 480-505, 1930.
Ibid., **98**, 279-295, 1931a.
Ibid., 296-310, 1931b.
Quart. Rev. Biol., **7**, 1-26, 1932.
Biol. Rev., **11**, 49-82, 1936.

Tarr, H. L. A. *J. Fish. Res. Bd. Can.*, **5**, 211-216, 1941.
Fish. Res. Bd. Can., Pac. Prog. Rept., No. 52, 24-26, 1942.

Proteins in Fish Muscle. IV. Denaturation by Salt[1]

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ABSTRACT

Study of the denaturation of cod muscle proteins by sodium chloride shows that the myosin fraction is denatured when a critical salt concentration, about 8 to 10 per cent in the muscle, is reached. Paralleling the rapid denaturation, a sudden increase of salt uptake and of moisture loss occurs.

INTRODUCTION

The proteins present in cod muscle have been investigated by Reay (1933, 1935), Reay and Kuchel (1937), and by Dyer *et al.* (1950). The last paper summarizes previous work. A globulin, myosin, makes up about 75 per cent of the total protein present. Smaller amounts of globulin X, and the albumins, myogen and myoalbumin are present.

Szent-Gyorgi (1947, 1948) has further fractionated myosin into two components, actin and "myosin". These two components combine to form actomyosin, which is the myosin of earlier studies, and this latter term will be used in the present paper. Dubuisson (1946) has obtained two fractions, α myosin and β mysin, by electrophoresis and by ammonium sulphate fractionation. The β myosin seems to be identical with Szent-Gyorgyi's "myosin" and α myosin with actomyosin.

Since almost complete extraction of the protein is obtained by the method in use in our laboratory (Dyer *et al*, 1950), it appears that the myosin obtained is identical with actomyosin. Any adenosinetriphosphate present originally would have been decomposed in the muscle samples as used, and the "myosin" and actin would thus have combined in the actomyosin complex.

Protein denaturation is a subject open to considerable controversy. Usually the term is applied in an arbitrary manner and has been used specifically in our investigations to denote change in solubility of the protein fractions. Myosin has been shown (Dyer, unpublished) to be the most labile of the protein fractions present in fish muscle. On freezing and storage, the myosin in frozen fish is denatured and becomes almost completely insoluble in salt solutions. This accounts wholly for the decrease in soluble protein in frozen fish.

Some aspects of the effect of salt on fish muscle proteins can be seen from the work of Snow (1950b), who found that the denaturation of myosin by freezing was affected by the presence or absence of salt, the freezing temperature and the type of salt present. The denaturation was found to be related to the

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physical state, no denaturation occurring when the protein was in the sol state. In salt solutions, denaturation occurred only below the eutectic temperature, when the myosin would be precipitated as a gel.

The penetration of sodium chloride into, and the removal of water from, fish muscle has been shown (Taylor, 1922; Dyer, 1942) to be faster at higher temperatures. Recently Fougere (unpublished) concluded that there is a critical concentration of sodium chloride in the flesh at which the initial process of uptake of both salt and water changes to a process of water removal accompanied by continuation of salt uptake. This critical concentration was found to be in the vicinity of 9 per cent sodium chloride.

In this paper the effect of immersing cod muscle in various concentrations of sodium chloride brine at several temperatures on protein solubility was studied.

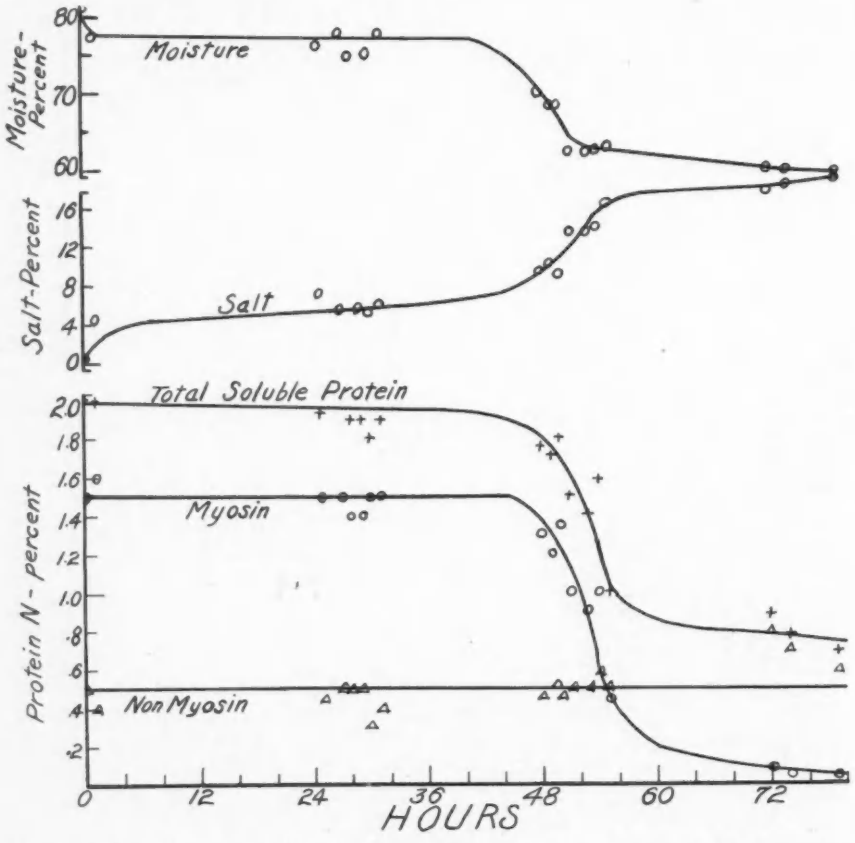

FIGURE 1. Loss of water, uptake of sodium chloride and changes in total soluble protein, myosin protein, and non-myosin protein, in cod fillets immersed in saturated sodium chloride at 0°C.

EXPERIMENTAL

Cod fillets as uniform in size as possible (2 cm. in thickness) were immersed in saturated sodium chloride brine at 0°, 5° and 20°C. The brine was stirred occasionally and kept saturated by addition of salt. Samples of muscle were taken for analysis at intervals as shown in Figure 1.

Protein estimations were made by the biuret method developed in this laboratory (Snow, 1950a). Samples of 22.5 g. fish muscle were made up to 450 ml. with 5 per cent sodium chloride solution for extraction of soluble protein according to Dyer *et al.* (1950). The myosin concentration was determined, as in the latter paper, by precipitation by dilution. For analysis the precipitated

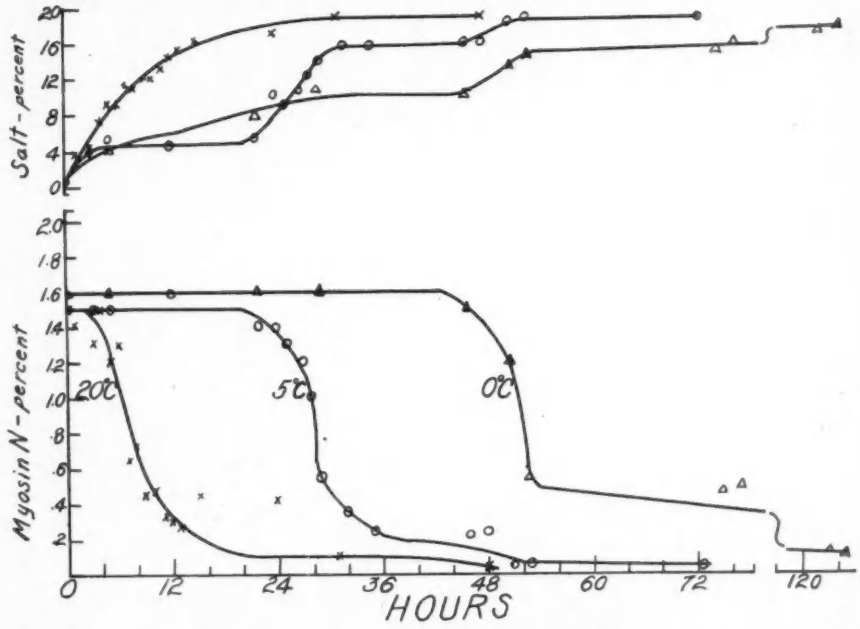

FIGURE 2. Changes in soluble myosin and salt content of cod fillets immersed in saturated brine at temperatures of 0°, 5°, and 20°C.

myosin was redissolved in either N sodium hydroxide or 5 per cent sodium chloride. The salt content of the samples was determined by the argentometric procedure of Dyer (1943) using dichlorofluorescein as an adsorption indicator. Moisture was estimated by drying to constant weight at 105°C. The results of several experiments are summarized in Figures 1 and 2. It is seen that the total soluble protein remains constant until a critical salt content of about 8 to 10 per cent is reached in the fillet. A sudden decrease in the solubility of the protein then occurs. This is parallelled by an equally rapid rise in salt uptake and decrease in water content, both of which approach equilibrium levels. The decline in total soluble protein is accounted for wholly by the decrease in soluble

myosin, the non-myosin protein remaining practically constant. Thus, it is the myosin fraction which is denatured, while the albumin remains unaltered. The effect of salt is, therefore, similar in this respect to freezing denaturation (Snow, 1950b).

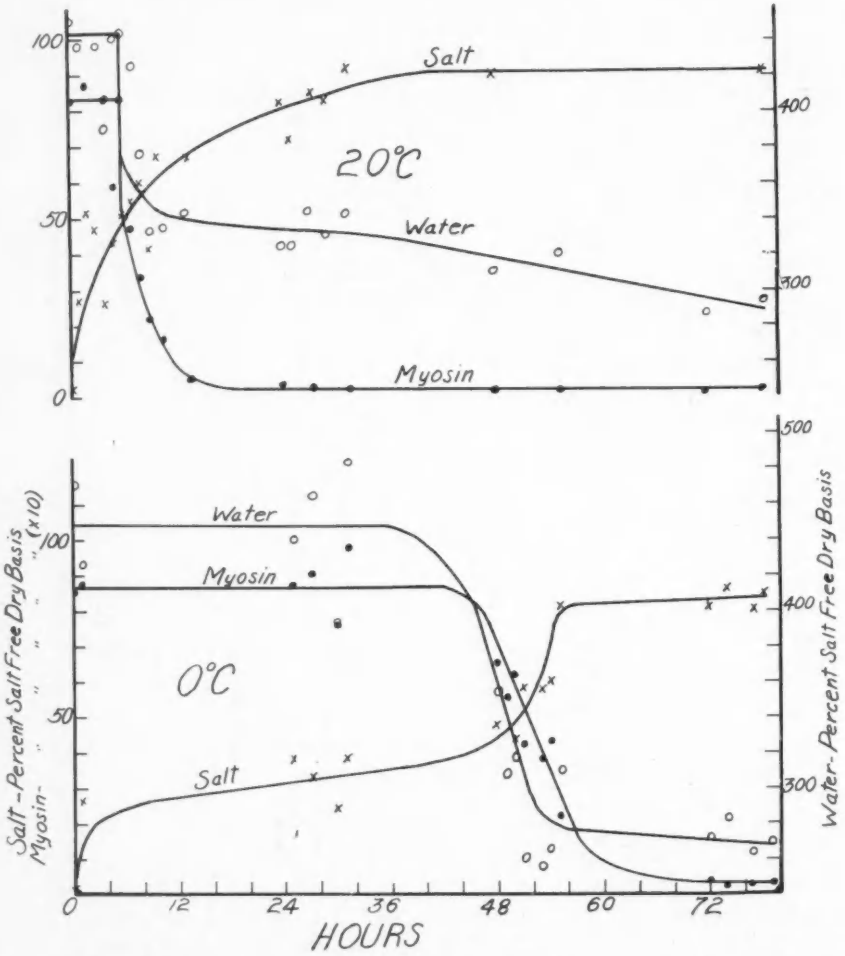

FIGURE 3. Loss of water (o), uptake of sodium chloride (x), and changes in soluble myosin (•), expressed as percentage of the salt-free dry weight, in cod fillets immersed in saturated sodium chloride at 0° and 20°C.

The fact that the large changes in salt and in water content occur at the same time as the myosin denaturation emphasizes the major change which denaturation of the myosin entails.

As the salt penetrates the tissue, it dissolves in the water associated with the protein. In solutions of salt concentration of about 2 to 5 per cent, myosin swells very strongly, and the water in the tissues becomes more firmly bound to the myosin micelles. But it still retains its salt solvent property, and more salt enters until at a concentration of about 10 per cent, as shown in Figures 1 and 2, the protein suddenly denatures. It loses its gel structure rapidly and releases a large part of the solution formerly held by the myosin gel. Thus, suddenly

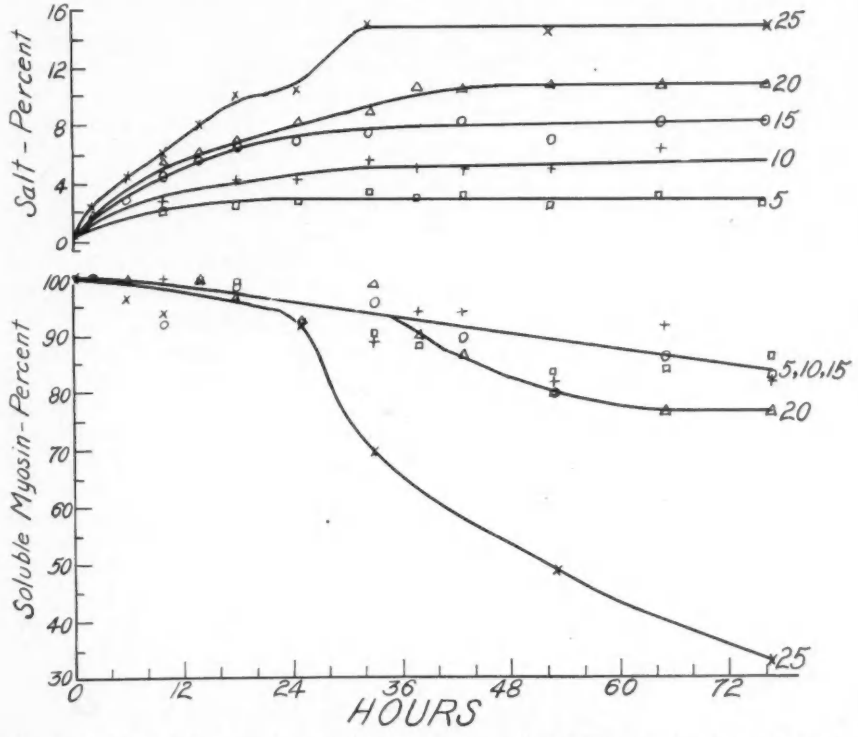

FIGURE 4. The soluble myosin and salt concentrations of cod fillets immersed in brines of sodium chloride concentrations of 5, 10, 15, 20, and 25 per cent at 0°C.—the myosin concentration is expressed as percentage of the initial concentration.

this water becomes free and rapidly diffuses out of the tissue into the more saturated brine outside. This accounts for the drop in moisture content at this point. The salt continues to penetrate, and indeed its rate of diffusion is accelerated by the collapse of the gel structure and the opening of the myosin micellar complex on denaturation. This acceleration of moisture removal and of salt diffusion is better shown in Figure 3 where the salt and water contents have been expressed as percentages of the salt-free dry residue. It is evident that the loss in moisture and the salt uptake is not the result of calculation.

The effect of temperature is shown in Figure 2. The critical salt concentration is reached in a much shorter time at the higher temperatures, and denaturation of myosin again occurs which parallels the changes in rate of salt uptake and water removal. The critical salt level appears to be unaffected by the temperature. The chief effect of raising the temperature is the faster penetration of salt ions, as discussed above.

The results of similar experiments using salt baths of 5, 10, 15, 20 and 25 per cent sodium chloride at a temperature of 0°C are shown in Figure 4. The fillets salted in 25 per cent sodium chloride brine reach an equilibrium salt concentration of 15 per cent salt in about 36 hours. Rapid denaturation of myosin begins when the salt content reaches about 10 per cent, but is slower than with saturated solutions. The salt penetration rate increases at this point also, parallelling the former experiments. In the 20 per cent salt bath, denaturation occurs when the salt content of the fillet reaches about 10 per cent, but does not proceed as far as with the 25 per cent or saturated salt brines. With 15, 10, and 5 per cent salt baths, the critical salt concentration in the tissue is not reached and almost no denaturation occurs.

Thus in brines of salt concentration below saturation, the salt content of the fillet and the degree of denaturation are related to the salt concentration of the bath. The water concentration inversely follows the salt concentration. In the above experiment the water concentrations at equilibrium were 69, 73, 77, 80 and 83 per cent in the 25, 20, 15, 10 and 5 per cent salt baths respectively.

It is realized that data from other criteria of denaturation are needed to supplement the data on solubility. Snow (unpublished) has found that myosin extracted from frozen fish does not have a larger number of free thiol groups than myosin extracted from fresh fish. This indicates that when myosin retains its salt solubility, it also shows no change in free sulphydryl groups, and by these criteria is not partially denatured; that is, the individual myosin unit is either denatured or is unchanged. On the other hand, Edsall and Mehl (1940) have shown that a variety of inorganic salts can abolish the double refraction of flow of myosin and increase its intrinsic viscosity at the same time.

In these experiments with fillets, difficulty was encountered in obtaining representative samples. The variation in thickness of different fillets and of parts of the same fillet led to differing rates of uptake of salt, moisture removal and myosin denaturation. Thus considerable scattering of the points is evident in the graphs.

SUMMARY

Study of the effect of strong salt solutions on the denaturation of fish muscle proteins in situ has shown that the myosin fraction only was denatured, the albumin portion not being affected. The myosin denaturation, as measured by salt solubility, occurred at a definite salt concentration, about 8 to 10 per cent sodium chloride in the tissue, and rapidly reached completion. The critical concentration was independent of temperature and of salt concentration of the suspending medium: these factors affected only the rate of attainment of the critical salt level. Denaturation was complete in about 13 hours at 20°C and about 70 hours at 0°C.

The moisture content decreased parallel with the sudden denaturation of the myosin, owing to the release of water imbibed by the myosin gel, and the rate of salt uptake increased.

REFERENCES

DUBUISSON, M. Monograph No. 5, VII Congress of Biological Chemistry, 199-207. Paris: Masson & Co., 1946.

DYER, W. J. *Fish. Res. Bd. Can., Atl. Prog. Rept.*, No. 33, 3-7, 1942.

DYER, W. J. *Ind. Eng. Chem. Anal. Ed.*, **15**, 439-440, 1943.

DYER, W. J., H. V. FRENCH, AND J. M. SNOW. *J. Fish. Res. Bd. Can.*, **7**, 585-593, 1950.

EDSALL, J. T., AND J. W. MEHL. *J. Biol. Chem.*, **133**, 409-429, 1940.

REAY, G. A. *J. Soc. Chem. Ind.*, **52**, 265-270T, 1933.

REAY, G. A. *J. Soc. Chem. Ind.*, **54**, 96-98T, 1935.

REAY, G. A., AND C. C. KUCHEL. *Rep. Food Inv. Bd. Gr. Brit.*, **1936**, 93-95, 1937.

SNOW, J. M. *J. Fish. Res. Bd. Can.*, **7**, 594-598, 1950a.
Ibid., 599-607, 1950b.

SZENT-GYORGYI, A. Chemistry of muscular contraction. 150 pp. New York: Academic Press, 1947.

SZENT-GYORGYI, A. Nature of life. 91 pp. New York: Academic Press, 1948.

TAYLOR, H. F. *Rept. U.S. Comm. Fish.*, **1922**, App. II, 1-22, 1922.

Effect of Storms on the Water Conditions in the Magdalen Shallows

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ABSTRACT

Extreme variations in temperature and salinity have been observed at depth, during the summer months, off North Rustico, P.E.I., and Cheticamp, N.S. These were correlated with wind force and direction, controlling factors of internal adjustment of water layers according to Ekman's theory of wind-driven currents.

Organisms which cannot tolerate these sudden changes in temperatures and salinities will not form important groups in populations along the margins of the Magdalen Shallows.

INTRODUCTION

Atmospheric circulation is generally associated with areas of high and low barometric pressure, semi-permanently established or moving slowly in an easterly direction. The latter is indicated in part by the passage of tropical and extra-tropical cyclones, atmospheric disturbances commonly called storms. The passage of these storms over a large body of water like the Gulf of St. Lawrence, subjects the waters to two extraneous forces:

(*a*) the frictional driving force of strong winds, and

(*b*) the forces of intense pressure gradients.

Ekman's theory of wind-driven currents has been applied successfully to the waters of the Scotian Shelf (Hachey, 1937), and the widespread effects of tropical cyclones on these waters have also been demonstrated (Hachey, 1935). The general effects of winds and pressure gradients on tides and currents in the Gulf of St. Lawrence have been summarized (Dawson, 1913), but the only information available on the internal adjustments in the water masses has been a short note on the waters off the Gaspé Coast (Tremblay and Lauzier, 1942).

Intensive oceanographic investigations of the Gulf of St. Lawrence were initiated in 1945 by the Atlantic Herring Investigation Committee, and shore stations off North Rustico, Prince Edward Island, and Cheticamp, Nova Scotia, were established for the purpose of observing temperatures and salinities of the water columns at regular intervals. At times, large variations in the salinity and temperature were observed at certain depths. These observed variations may be readily correlated with the passage of cyclonic disturbance in the atmosphere.

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THE WATERS OF THE MAGDALEN SHALLOWS IN SUMMER

The location of the points of observation are shown in Figure 1. These points are particularly exposed in the south-western area of the Gulf, which area has been termed the Magdalen Shallows.

Typical August water conditions in the Magdalen Shallows are shown in sections in Figure 2, one section extending from North Rustico to Bradelle Bank and one from Magdalen Island to Cheticamp (Figure 1). In particular, a surface layer of water of a temperature greater than 15.0°C. and a salinity of 28.0 ‰ or less extends to a depth of approximately 10 metres. Underlying this surface layer is a thermocline with a temperature gradient of approximately one degree per metre, and a corresponding salinity gradient of 0.2 ‰ per metre. Underneath the thermocline is a body of water of temperatures lower than 5.0° and as low as −0.5°, and of salinities higher than 30.0 ‰ and as high as 32.4 ‰. The surface layer as well as the thermocline and the halocline are deeper along Prince Edward Island and Cape Breton Island than over Bradelle Bank and around Magdalen Island. This is shown by slanting isotherms and isohalines.

This stratification of the waters is a feature of the summer months in the Magdalen Shallows, the surface layer increasing in thickness as the season progresses. As a result of the presence of a marked thermocline, the surface layer is very stable with respect to the underlying layers and therefore considerable resistance is offered to external forces which would tend to produce vertical mixing. The reaction, therefore, to external forces is a matter of internal adjustments between layers.

Periodical Observation at North Rustico, 1945 to 1949

Observations on the water column off North Rustico, P.E.I., were made weekly during the spring, summer and autumn months throughout the years 1945 to 1949. During the summer months particularly, extreme variations in the temperature and salinity of the water column were to be observed at depth. These variations are illustrated in Figure 3 (*a*) by the observations throughout the months of June, July and August of 1949. Throughout this period, at 20 m. the temperature varied from a low of 6.9° late in June to a high of 17.0° in late July, while the salinity varied from a high of 30.4 ‰ in late June to a low less than 27.8 ‰ in late July. Temperature varied by as much as ten degrees within a week, while the corresponding salinity variations were more than 1.5 parts per thousand. In particular, it will be noted that increasing temperatures were associated with decreasing salinities and vice versa.

Combined Observations at North Rustico and Cheticamp, 1947 to 1948

Weekly observations were made on the water column at North Rustico, P.E.I., and Cheticamp, N.S., during the summer seasons of 1947 and 1948. In several cases, abrupt changes in temperature and salinity were observed to take place at one or other of the stations or approximately at the same time at

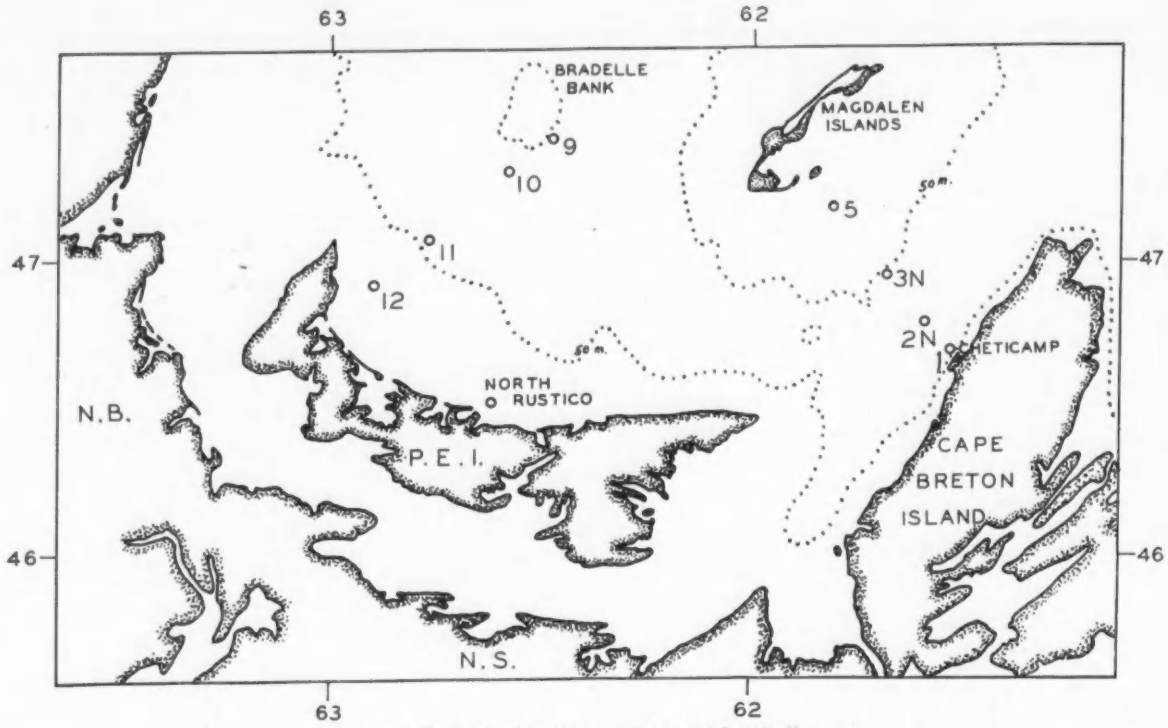

FIGURE 1. Location of stations on the Magdalen Shallows.

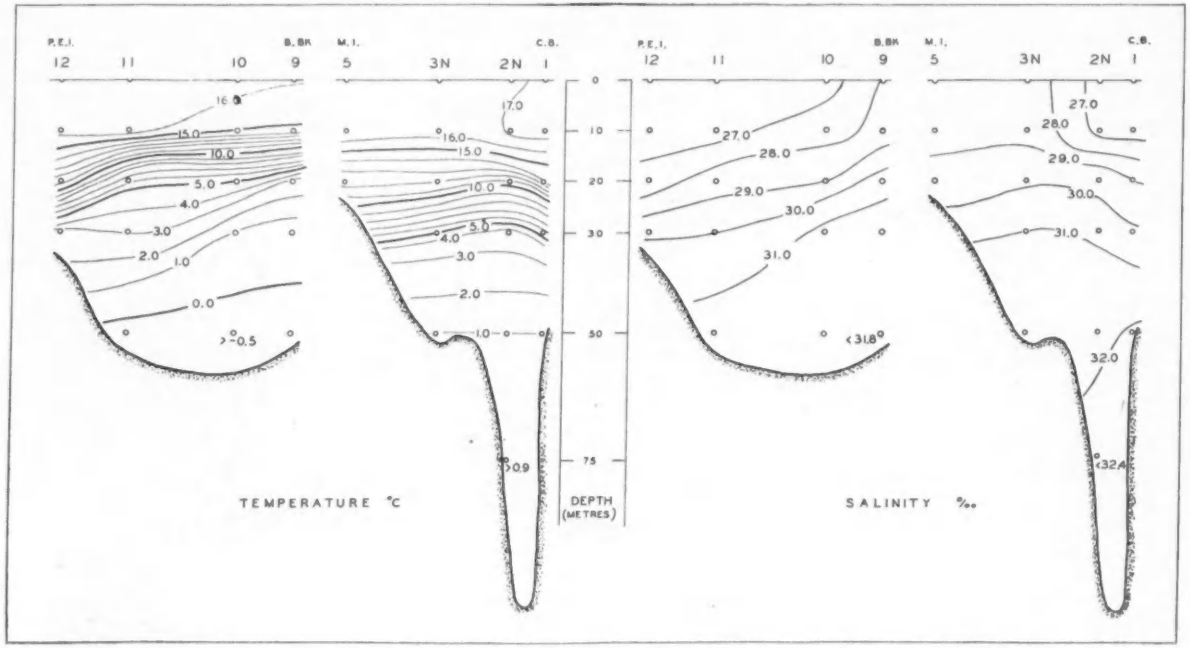

FIGURE 2. Typical August temperature and salinity distribution in the Magdalen Shallows (Aug. 29-30, 1947).

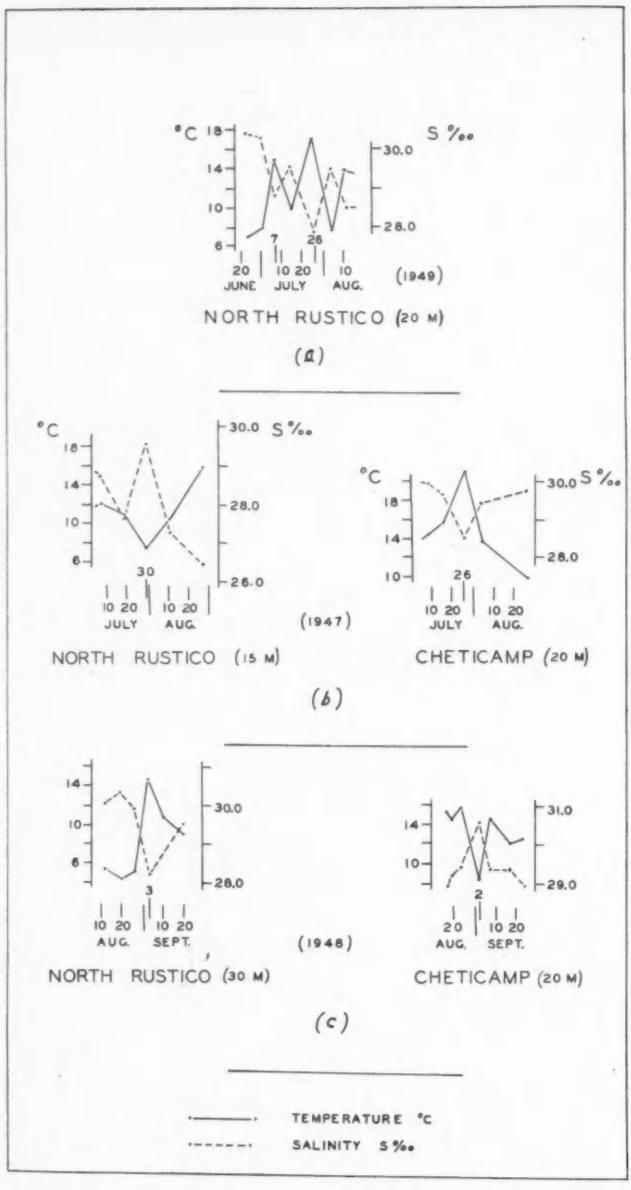

FIGURE 3. Temperature and salinity variations at North Rustico, P.E.I., and Cheticamp, N.S.

both stations. Two out of seven cases are plotted in Figures 3(*b*) and 3(*c*). The depths are chosen to illustrate the maximum variations that occurred.

These observations at North Rustico and Cheticamp again demonstrate that large variations in temperature and salinities occur at particular depths of the water columns. It is further demonstrated that when these large variations are observed:

(*a*) increasing temperatures were associated with decreasing salinities and vice versa,

(*b*) increasing temperatures and decreasing salinities at North Rustico were associated with decreasing temperatures and increasing salinities at Cheticamp, and

(*c*) decreasing temperatures and increasing salinities at North Rustico were associated with increasing temperatures and decreasing salinities at Cheticamp.

The major internal disturbances in the water columns at North Rustico in 1947 were observed preceding July 30th, when the temperature at 15 metres decreased from approximately 11°C. on July 20th to 7.5°C. on July 30th and with a corresponding increase in salinity from 27.6‰ to 29.6‰. During this same period, the temperature at 20 m. off Cheticamp increased from 15.5°C. to 21.0°C. with corresponding decrease in salinity from 29.6 ‰ to 28.5 ‰.

In 1948 the major internal disturbances were observed at North Rustico preceding September 3rd when the temperature at 30 m. increased from 5.0°C. on August 27th to 14.5°C. on September 3rd, with corresponding decrease in salinity from 29.9 ‰ to 28.2 ‰. During this same period the temperature at a depth of 20 m. in the water column at Cheticamp decreased from 16.0°C. to 8.4°C. with a corresponding increase in salinity from 29.4 ‰ to 30.6 ‰.

Correlation with Wind Force and Direction

In order to correlate major internal disturbances in the water columns at North Rustico and Cheticamp with wind strengths and direction, the weather maps as prepared by the Public Weather Office in Halifax were consulted. It was found that these disturbances were associated with winds of at least 15 miles per hour, blowing for at least two days. Winds from the N and NW were associated with increasing temperatures and decreasing salinities at North Rustico, and decreasing temperatures and increasing salinities at Cheticamp. On the other hand, southerly winds were associated with decreasing temperatures and increasing salinities at North Rustico and increasing temperatures and decreasing salinities at Cheticamp.

Theoretical Considerations

Figure 4 is illustrative of a theoretical section in the Magdalen Shallows between North Rustico, P.E.I., and Cheticamp, N.S., consisting of two water layers of different temperature and salinity characteristics and separated by a steep thermocline. In 4(*a*) the layers are of uniform thickness, and the body of water does not show any motion normal to the section. According to Ekman's theory of wind-driven currents, there is, in the northern hemisphere, a net

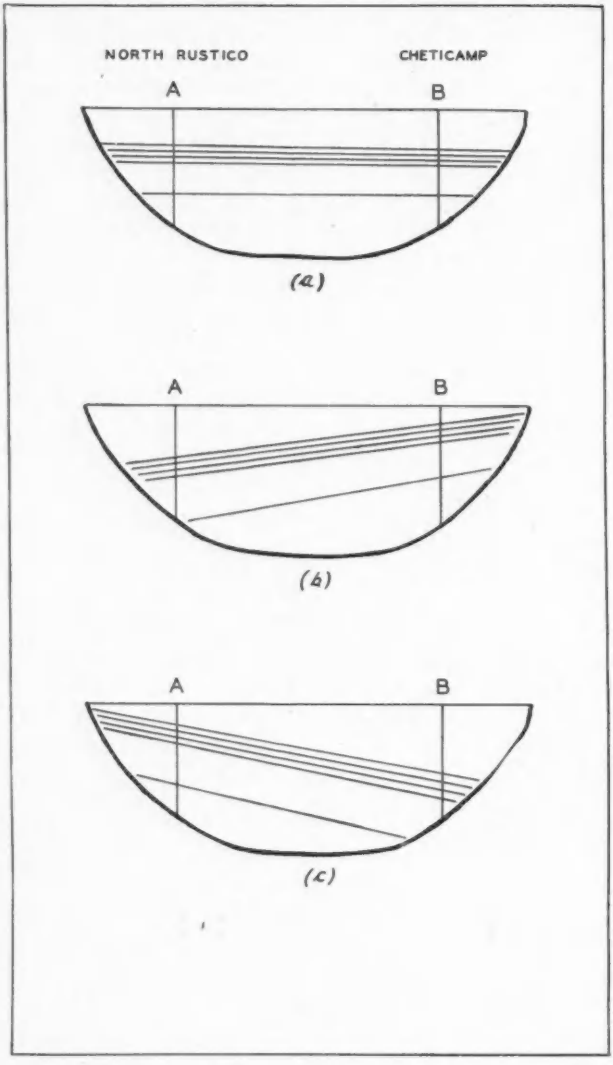

FIGURE 4. Theoretical section between North Rustico and Cheticamp under different conditions: (*a*) no motion, (*b*) northerly wind direction (out of the paper), (*c*) southerly wind direction (into the paper).

transport of water to the right of the direction of the wind acting upon the sea surface. In the presence of a boundary parallel to the wind direction, there will be piling up or removal of water depending on the location of the boundary to the right or left of the wind direction. If the theoretical section is a west-east section and the surface waters are subjected to a frictional force of a wind blowing from a northerly direction (out of the paper) the combined effect of the wind stress and the earth's rotation would bring about a distribution of waters as shown in Figure 4(*b*), in piling up water on the right of wind direction and removal on the left. If, on the other hand, the surface waters were subjected to a wind blowing from a southerly direction (into the paper), the rearrangement of the water layers would similarly be as illustrated in Figure 4(*c*).

Further, if the observation points were A and B and consideration were given to the variations in temperature and salinity as observed on the bottom, or at fixed depths, under northerly winds as in Figure 4(*b*), the surface layer would thicken at point A, bottom temperatures would increase and salinities would decrease. At point B, the surface layer would decrease in thickness, the bottom temperatures would decrease and the salinities would increase. Under southerly winds as in Figure 4 (*c*), the reverse situations would be observed. Hence we may look upon the actual observations at North Rustico, P.E.I., and Cheticamp, N.S., as reflecting internal adjustments throughout the waters of the Magdalen Shallows.

DISCUSSION

On a shelving shore, the full significance of the foregoing changes in thickness of the water layers may be appreciated from an ecological point of view. Depending upon the depth and the slope, there must be a wide band of sea bottom in the Magdalen Shallows which is subjected to sharp and intensive variations in temperatures and salinities as have been illustrated in the foregoing. It becomes evident that organisms which cannot tolerate these sudden changes in temperatures and salinities will not form an important group in the populations in certain areas of the Magdalen Shallows.

ACKNOWLEDGMENT

The writer wishes to extend his thanks and appreciation to H. B. Hachey for his interest and assistance in preparing this paper.

REFERENCES

Dawson, W. B. The current in the Gulf of St. Lawrence. Ottawa: Department of the Naval Service, 1913.

Hachey, H. B. The effect of a storm on an inshore area with markedly stratified waters. *J. Biol. Bd. Can.*, **1**(4), 227-237, 1935.

Ekman's theory applied to water replacement on the Scotian Shelf. *Proc. Nova Scotia Inst. of Science*, **19**, 264-276, 1937.

Tremblay, J. L., and L. Lauzier. Explication de minimums de température des eaux de surface à Grande Rivière, en juillet, 1940. *Ann. de l'Acfas.*, **8**, 76, 1942.

The Lake Whitefish in Kerr Lake, New Brunswick

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(*Received for publication March 3, 1951*)

ABSTRACT

Coregonus clupeaformis was taken in a small lake of southern New Brunswick. Although the species had not been known to occur in this region, this population is evidently indigenous. Fish of ages III-V were taken, the largest being 252 mm. in fork length.

INTRODUCTION

The lake whitefish, *Coregonus clupeaformis* (Mitchill), is widely distributed in Canada (Dymond, 1947). In New Brunswick the species has long been known to occur in the St. John river system (Perley, 1852; Cox, 1896), but elsewhere in the Maritime region its distribution has been poorly defined. Dymond (1947) lists Lunenburg and Yarmouth Counties for distribution of this whitefish in Nova Scotia and suggests that it was possibly introduced into those areas. Two specimens of whitefish (30.9 cm. notch length, 328 gm.; 28.7 cm., 270 gm.) taken with a gill-net in the Tusket River, Yarmouth County, by Fishery Warden H. C. Wood on April 10, 1951, and submitted to the author for examination, proved to be *C. clupeaformis*. In neighbouring Maine, Everhart (1950) reports that recent surveys found the species only in Moosehead Lake, although an earlier publication by Kendall (1914) gives a wider distribution in that State.

On July 3, 1945, Mr. C. Lowery found a specimen of *C. clupeaformis* about 20 cm. in length on the shore of Kerr Lake, Charlotte County, New Brunswick. Over the night of July 12-13, 1950, 38 additional specimens were captured in this lake by a gill-net. Since it is not planned to make further investigation of the Kerr Lake population, the data at hand are presented as a contribution to the biology of the species in a region where it has been little studied.

KERR LAKE

As shown in Figure 1, Kerr is one of five lakes that are tributary to Bocabec River—a short system flowing into Passamaquoddy Bay. Morphometric features of the lake are given in Table I.

The water of the lake becomes stratified in summer, but the volume of the hypolimnion in which serious depletion of the dissolved oxygen may occur has

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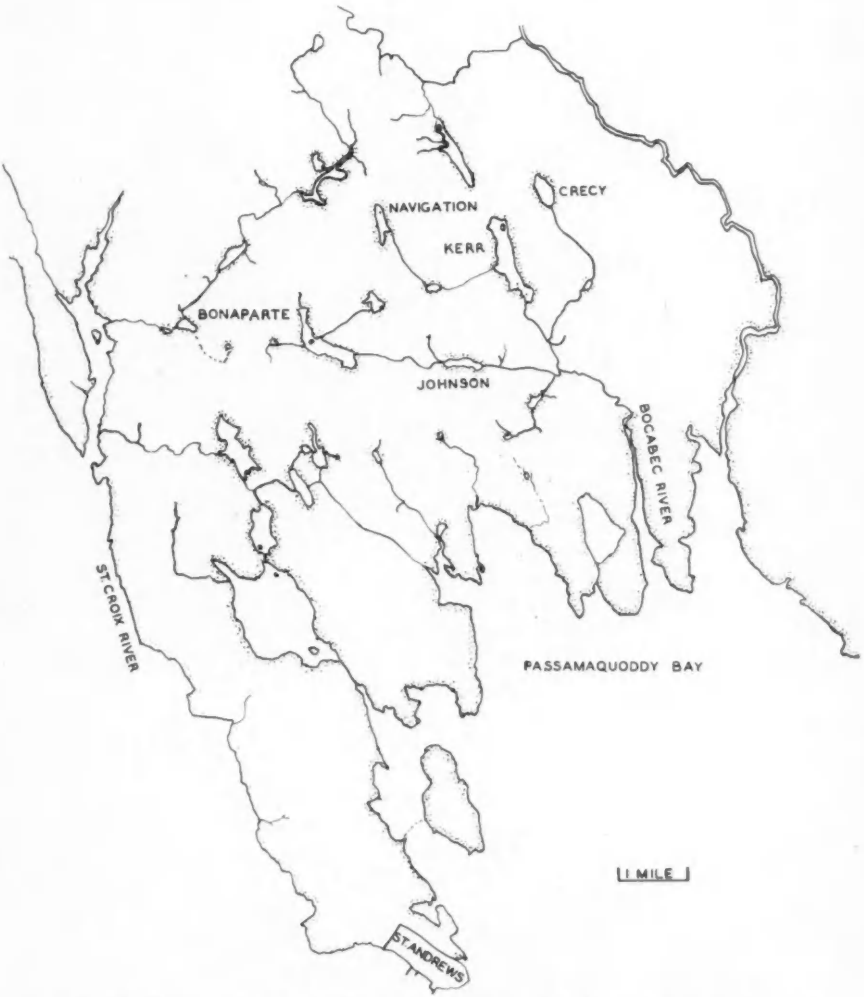

FIGURE 1. Map showing the location of Kerr Lake, Charlotte County, New Brunswick.

not been found to represent more than 2 per cent of the total for the lake. Summer water temperatures are illustrated by the following data:

	Surface °C.	Bottom	Mean for the lake
July 22, 1941	21.4	14.0	20.2
July 7, 1942	21.9	11.6	20.0

The water in the lake is soft. The half-bound carbon dioxide was 6.8 and 6.3 ml. per litre at the surface and bottom (11 m.) respectively on July 28, 1943, and the pH values (bromthymol blue) 6.8 and 6.0 for the same depths.

TABLE I. Morphometry of Kerr Lake.

Area	73.0 ha., 180.3 ac.
Maximum depth	12.0 m., 39.4 ft.
Mean depth	4.7 m., 15.4 ft.
Volume	3.41×10^6 cu. m., 120.43×10^6 cu. ft.
Perimeter	5.96 km., 3.70 mi.
Volume development	1.18
Shore development	1.99

CAPTURE OF THE WHITEFISH

The whitefish were taken in a gill-net of 2-inch (5.1 cm.) stretched mesh, 6 ft. (1.8 m.) deep and 25 yd. (22.9 m.) long, sunk to the bottom in about 7.5 m. of water. All of the specimens were found enmeshed not more than 2 ft. (0.7 m.) from the bottom of the net. Two other nets were also set at the same time, but took no whitefish—one of 2¾-inch (7.0 cm.) stretched mesh to the bottom in 9 m. of water and the other of 3-inch (7.6 cm.) stretched mesh in shallower water of half that depth. The results suggest, assuming that the meshes of the latter nets were not too large to capture them, that the whitefish were avoiding the oxygen-depleted deep water in the one situation and the warm water in the other. (On July 7, 1942, there was only 2.47 ml. of dissolved oxygen per litre at 10 m., and a temperature of 20.2°C. at 4 m., while at 7.5 m. the oxygen content approximated 5 ml. per litre and the temperature 18°C.)

No whitefish were captured from mid-morning until late afternoon, July 12, 1950, a day with an overcast sky. Of the 38 individuals that were netted during the night, ten were so mutilated by eels as to be useless for study.

CHARACTERISTICS OF THE FISH

METHODS

Scales for age determination were taken from the area just posterior to the dorsal fin and above the lateral line. Three separate readings were made by Miss E. Haskell on the scales from each fish. The scales were not mounted, but were placed upon a glass slide and bathed in a detergent (Aerosol) before reading under a binocular microscope.

Morphological measurements were taken after the fish has been preserved in formalin for four months. Kennedy (1943) was largely followed for method

in making the measurements listed in Table II. Lengths were recorded to the nearest millimetre on a steel rule, and weights to the nearest 0.5 gram on a torsion balance. Other body measurements were made with sharp-pointed dividers to the nearest 0.5 millimetre. Methods of counting fin rays were those outlined by Hubbs and Lagler (1941), which involve the principal but not the rudimentary rays.

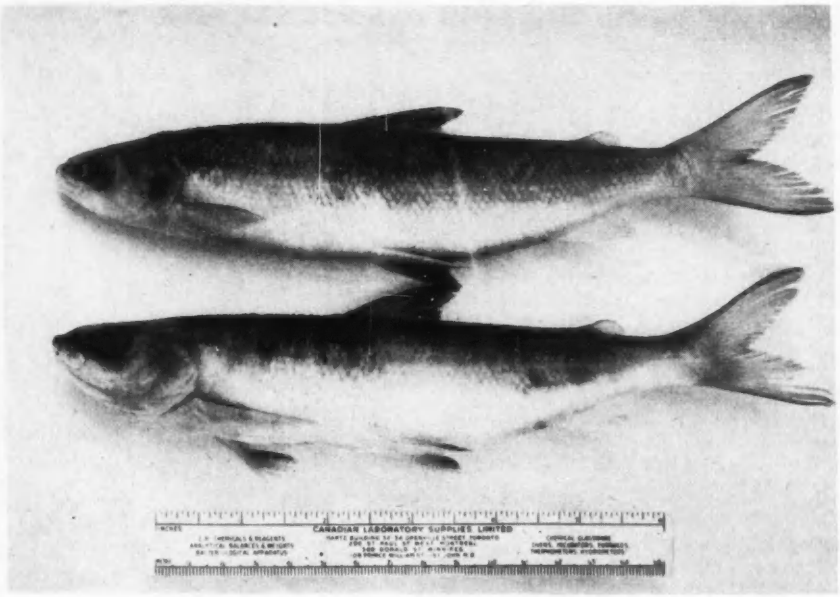

FIGURE 2. Whitefish from Kerr Lake. Photo by G. F. M. Smith.

SEX AND MATURITY

There were 19 males among the 28 whitefish that were examined. The gonads were well developed in both sexes, and there appeared little doubt that they would have spawned in the fall of 1950. In this connection it is pertinent to note that no whitefish entered traps which were maintained at the mouths of the tributary streams to Kerr Lake to capture trout during the late summer and fall of 1941, which observation suggests that the whitefish spawn in the lake proper.

MORPHOLOGICAL DATA

Coregonus clupeaformis is a variable species for which a number of subspecies have been described (Koelz, 1931). However, no attempt has been made to refer the specimens from Kerr Lake to a particular subspecies, since, even if it were desirable, the small number does not warrant that exhaustive treatment.

Various morphological data we have presented in Table II. Kennedy (1943) made a careful study of dwarf and normal whitefish populations in Lake Opeongo, Ontario, and found no definite difference in body proportions between the two groups when mature fish were considered. Similarly, except possibly for snout length, it may be shown that the whitefish from Kerr Lake exhibit only minor variations from the mature Opeongo fish in body proportions. The numbers

TABLE II. Morphological data on whitefish from Kerr Lake (length measurements are in millimetres).

Sex	Fork length	Standard length	Weight (gm.)	Head length	Snout length	Eye diameter	Anal fin length	Pectoral fin length	Caudal peduncle depth	No. of gill rakers	Rays in dorsal fin	Rays in anal fin	Scales on lateral line
						Age 3 +							
♀	190	176	83.0	35.5	10.5	8.5	23.0		13.0	28	11	11	70
♀	191	176	86.0	39.5	11.0	9.5	22.0	30.5	13.5	27	11	10	68
♀	200	184	90.0	41.5	11.0	10.0	23.5	33.0	14.5	26	10	9	
♀	202	188	94.5	40.0	11.0	10.0	25.5	34.5	13.5		11	10	71
♀	206	190	99.5	44.0	12.0	10.0	25.0	36.0	14.5	27	11	10	
♂	190	175	78.0	38.0	10.0	9.5	22.5	34.0	13.5	26	11	11	66
♂	203	189	91.0	41.5	11.5	9.5	23.5	30.5	14.0	27	11	10	
♂	209	194	89.5	42.5	12.0	9.5	24.0	35.5	14.0	26	11	10	
♂	210	194	84.0	42.5	11.5	10.5	27.0	36.0	14.0	26	11	11	
♂	214	199	96.5	43.5	12.5	10.5	28.0	36.5	14.0	27	11	11	
Av.	201.5	186.5	89.2	40.9	11.3	9.8	24.4	34.1	13.9	26.7	10.9	10.3	
						Age 4 +							
♀	202	187	92.0	41.0	10.5	10.0	25.0	32.5	13.5	26	11	11	
♀	206	191	97.5	42.0	11.0	9.5	25.0	35.0	14.0		10	10	
♂	197	182	84.0	39.5	11.0	8.5	24.5	31.5	14.0	27	11	11	72
♂	201	185	85.0	40.5	11.5	9.0	20.0	33.0	14.5	28	11	10	
♂	203	188	96.5	42.0	11.0	9.5	27.0	38.5	14.0	28	11	10	
♂	205	190	92.0	40.0	10.5	10.0	27.0	34.5	14.0	26	10	11	68
♂	206	191	97.0	40.5	11.0	9.0	20.0	33.0	14.5	26	11	9	69
♂	207	192	94.5	43.0	11.5	10.0	25.5	37.0	14.0	25	11	11	
♂	209	193	95.5	43.5	12.0	10.5	26.5	39.5	15.0	27	9	10	
♂	212	197	103.0	44.0	12.5	10.5	28.0	39.0	14.5		11	11	
♂	213	197	93.0	44.0	12.0	10.5	28.0	37.5	14.5	28	11	10	69
♂	213	198	104.5	44.5	12.5	10.5	26.5	36.5	14.5	26	11	10	
♂	216	201	106.0	45.0	12.5	10.0	27.5	35.0	15.0	27	11	10	69
Av.	206.9	191.7	95.4	42.3	11.5	9.8	25.4	35.6	14.3	26.7	10.7	10.3	
						Age 5 +							
♂	252	233	175.0	52.0	14.0	12.0	31.0	42.0	17.5	27	11	11	69

of rakers on the first gill arch of the fish from the New Brunswick lake are intermediate, however, between those for the two groups studied by Kennedy. Also, the average number of scales in the lateral line of the Kerr Lake whitefish is definitely low for the species, but, because of the loss of scales in the net, a reasonably accurate count was possible only on ten specimens. Specimens of the whitefish from Kerr Lake are pictured in Figure 2.

AGE

The ages of 24 whitefish from Kerr Lake were determined. One individual, 252 mm. in fork length, was in its sixth year (5+) of age. Among the remainder, there were 13 specimens in their fifth (4+) and 10 in their fourth (3+) year. The average fork lengths of the latter year classes were 207 and 202 mm. respectively and the corresponding standard deviations (s) were 5.5 and 8.7

FIGURE 3. Scale of whitefish from Kerr Lake showing three annuli. Notch length 21.6 cm. Magnification x 25. Photo by Emma Haskell.

mm. The seemingly poor growth rate, as illustrated by the small difference in these means, is considered attributable to the selectivity of the gill-net whereby the sample was biased toward containing an unrepresentative proportion of the larger individuals in the 3+ year class on the one hand and the smaller fish in the 4+ group on the other. A scale is illustrated in Figure 3.

DISCUSSION

The recovery of the whitefish in Kerr Lake illustrates well how an important constituent of the fish fauna of even a small lake in an area that has long been settled may be overlooked until accidentally found by an interested party or until proper means are taken for its capture. Such a situation obtained at Kerr Lake notwithstanding that the lake had been subject to angling for many years, principally, however, for speckled trout, the angling for which would be unlikely to take whitefish. In recent years certain limnological observations have been made as well.

Presumably the whitefish is indigenous to Kerr Lake. Young speckled trout have been planted in the lake from time to time during the last twenty years from New Brunswick fish hatcheries, but the possibility of introducing whitefish inadvertently along with the trout is ruled out, since at no time have these establishments hatched or reared whitefish.

The species is not known to be present in neighbouring lakes. Our experiences at Kerr Lake suggest that exploration with suitable gear would disclose a wider distribution of the species in the lake districts of New Brunswick and Nova Scotia than is now realized.

LITERATURE CITED

Cox, Philip. Catalogue of the marine and fresh-water fishes of New Brunswick. *Bull. Nat. Hist. Soc. New Brunswick*, No. 13, 62-75, 1895.

Dymond, J. R. A list of the freshwater fishes of Canada east of the Rocky mountains. *Roy. Ontario Mus. Zool., Misc. Publ.*, No. 1, 1-36, 1947.

Everhart, W. Harry. Fishes of Maine. 53 pp. Augusta: State of Maine Dep. Inland Fish Game, 1950.

Hubbs, C. L., and K. F. Lagler. Guide to the fishes of the Great Lakes and tributary waters. *Bull. Cranbrook Inst. Sci.*, No. 18, 1-100, 1941.

Kendall, W. C. The fishes of Maine. *Proc. Portland Soc. Nat. Hist.*, **4** (1), 1-198, 1914.

Kennedy, W. A. The whitefish, *Coregonus clupeaformis* (Mitchill), of Lake Opeongo, Algonquin Park, Ontario. *Univ. Toronto Stud., Biol. Ser.*, No. 49 (*Publ. Ontario Fish. Res. Lab.*, No. 62), 21-66, 1943.

Koelz, Walter. The coregonid fishes of Northeastern America. *Papers Michigan Acad. Sci. Arts Letters*, **13** (1930), 303-432, 1931.

Perley, M. H. Reports on the sea and river fisheries of New Brunswick, 2nd. ed. 294 pp. Fredericton, 1852.

The Food and Growth of the Maskinonge (Esox masquinongy Mitchill) in Canadian Waters

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(Received for publication March 18, 1951)

ABSTRACT

Maskinonge from three regions were studied: the Lake of the Woods district in Ontario (called *Western*); the Kawartha Lakes and Georgian Bay district in Ontario (*Central*) and the St. Lawrence River district in Quebec (*Eastern*). Examination of 202 stomachs, 81 containing food, showed the maskinonge to be a general carnivore, preying mainly on fish over 150 mm. in length. The families Percidae, Catostomidae, Ameiuridae, Centrarchidae, Hiodontidae, Esocidae and Cyprinidae were represented, along with at least one *Cambarus*. The yellow perch, *Perca flavescens*, was the species eaten most frequently in all three regions. A common white sucker (*Catostomus commersonnii*) was found in a stomach of the hybrid *E. masquinongy* × *E. lucius*. Examination of the teeth of each specimen showed that they were being continuously replaced throughout the summer season.

Rate of growth varied with sex and locality. Females had a significantly faster rate of growth, both in length and in weight, than did males. Maskinonge from the Western Region were shorter and weighed less than fish of the same age from the other two regions. Maskinonge of the Eastern and Central Regions reached the legal size of 30 inches fork length during their fifth summer, but in the Western Region they did not attain this size until their seventh summer. The length-weight relationship appears to be the same in the Eastern and Central Regions, whereas maskinonge from the Western Region tended to be heavier than those of comparable lengths from the other two regions. In all regions the length-weight relationship was a straight line when plotted logarithmically, its slope being estimated as 3.26 in the Eastern Region. Sexual maturity occurs first between the ages of three and six years, or about 575 to 800 mm. fork length. Specimens of a *E. masquinongy* × *E. lucius* hybrid appeared to be infertile. They did not differ from the maskinonge specimens in their length-weight relationships but made faster growth than did the maskinonge from the same region.

INTRODUCTION

An investigation of the food and growth of the maskinonge in Canadian waters was carried out during the summers of 1946, 1947, and 1948. In Canada the maskinonge is found in bays of the Great Lakes, and in medium-sized lakes and larger rivers from the St. Lawrence River to the upper reaches of the Winnipeg River (Dymond, 1947). As it was impracticable to attempt to gather data on this fish over the entire extent of its known range, an investigator was sent for one summer to each of three general areas in Canada where maskinonge have been

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reported to be relatively abundant. These areas are indicated in Figure 1, and may be outlined as follows:

(i) WESTERN REGION. This district, from which specimens were examined in 1946, comprised the Kenora and Rainy River districts in north-western Ontario. In the course of the study the following lakes were visited: Little

FIGURE 1. A map of the Province of Ontario showing the regions included in this study: 1—Western Region; 2 and 3—Central Region; 4—Eastern Region.

Vermilion, Big Vermilion, Maskinonge, Eagle, Mud (Hooch), Cederbrough, Cedar, Clay, Class, Corner, Indian, and Fluke. Fish were also examined from the Sabascong and Whitefish Bays on Lake of the Woods. All of these waters drain into Hudson Bay.

(ii) CENTRAL REGION. This area was studied in 1947 and covered the Kawartha Lakes and Georgian Bay region of central Ontario. The waters visited

include Buckhorn, Lovesick, Pigeon, Rice, Scugog and Stoney Lakes, Indian River, Deer Bay, and Georgian Bay at Sans Souci. These waters all drain into the Great Lakes.

(iii) EASTERN REGION. This district, which was sampled in 1948, included the St. Lawrence River (and tributary waters) from the Ontario border to about 85 miles east of Montreal. The majority of fish examined were taken from Lake St. Francis, Lake St. Louis, and Lake of Two Mountains in the St. Lawrence River near Montreal.

The maskinonge is a comparatively rare fish and the area covered by the study was relatively large. It therefore seemed desirable to study the fish taken by anglers during the fishing season rather than attempt to gather specimens independently, since the latter method would not be likely to provide as many fish. The investigator stayed at fishing lodges and camps, and by being present when the fish were caught or brought back to camp, was able to record the necessary data, examine the stomach contents, and take scale samples. This was accomplished except when the fish had already been cleaned or was being kept for mounting. In 1948 in the Eastern Region, the Department of Fish and Game for the Province of Quebec offered a three-dollar reward to all anglers who reported catching a maskinonge and submitted the fish for examination. This served as an added stimulus and made possible the collection of data on a relatively large number of specimens at a reasonable cost.

FOOD

The maskinonge is noted as a voracious carnivore. Concerning this subject Eddy and Surber (1943) write: "Muskellunge are entirely carnivorous. Their food consists largely of suckers, perch and other fishes. Muskellunge reared in tanks at the University of Minnesota fed on water fleas for the first week of life, but thereafter refused such small fry and would feed only on small suckers and other fishes." Anderson (1948) states: "Upon examination of stomach contents, various items of aquatic fauna have been found, such as large walleyes, suckers, perch, minnows, and frogs. In the literature there are reports of investigators observing muskellunge feeding on young birds and young muskrats, swimming at the surface of waters."

A few noteworthy specific references have also been made. The *Toronto Evening Telegram* of September 27th, 1928, recorded the capture on a plug of a 34-pound maskinonge and stated that ". . . when the 'lunge was being cleaned, a muskrat was found in its stomach. It must have been freshly caught by the 'lunge for it was still kicking." Another such example was reported by Anderson (1948) from the stomach of a 50.5-inch, 43-pound maskinonge from Butternut Lake, Wisconsin. The taxidermist who opened the stomach of this fish found a partly digested, medium-size muskrat (*Ondatra z. zibethica*) and a freshly swallowed pied-billed grebe (*Podilymbus p. podiceps*).

MATERIALS AND METHODS

While gathering data in the Western and Central Regions, analysis of stomach contents had to be carried out in the field, since it was often impossible

to preserve and store the stomachs. Facilities for the identification of stomach contents were naturally not as complete under these circumstances as would be desired and time was more or less limited.

The stomachs taken in the Eastern Region were preserved in 5 per cent formalin. They were analysed at the Royal Ontario Museum of Zoology, where skeleton collections and literature were available to assist in making accurate identifications.

Results

WESTERN REGION. The stomachs of 59 maskinonge from the Western Region were examined. Of these 39 were empty, while 20 contained food in various stages of digestion (Table I). It would seem that maskinonge in this region fed almost entirely on fish, and largely on the yellow perch (*Perca flavescens*),

TABLE I. Stomach contents of 59 maskinonge from the Western Region (northwestern Ontario), 20 of which contained food.

Type of animal	Number of occurrences
Crayfish (*Cambarus* sp.)	1
Common white sucker (*Catostomus commersonnii*)	2
Yellow perch (*Perca flavescens*)	25
Unidentified Catostomidae	2
Unidentified Cyprinidae	2
Unidentified fish	4
TOTAL	36

as 69 per cent of the total number of occurrences were of this species. Only two other families of fish were identified in the stomachs from this district, Catostomidae and Cyprinidae. One stomach contained a single *Cambarus*.

Four stomachs of the hybrid *masquinongy* × *lucius* (Cameron, 1948) were also available for examination, and of these two were empty. One contained a common white sucker (*Catostomus commersonnii*) and the other was found to contain the remains of a fish, probably also a common white sucker.

CENTRAL REGION. Of the 27 maskinonge stomachs examined from this region, ten were empty. The contents of the remainder are summarized in Table II.

TABLE II. Stomach contents of 27 maskinonge from the Central Region (central Ontario), 17 of which contained food.

Type of animal	Number of occurrences
Yellow perch (*Perca flavescens*)	17
Rock bass (*Ambloplites rupestris*)	1
Bass (*Micropterus* sp.)	2
Unidentified Centrarchidae	3
Unidentified fish	1
TOTAL	24

It would appear that in this region too, the yellow perch provided the major part of the diet of the maskinonge, and in this case the number of multiple occurrences is comparatively low. Specimens of yellow perch made up 71 per cent of the food items. The only other identifiable family was the Centrarchidae, and no invertebrates were observed.

EASTERN REGION. One hundred and sixteen maskinonge stomachs from this region were examined, of which 72 were empty. The contents of the remaining 44 are summarized in Table III. A much greater variety of fish was found in stomachs from this area than from either of the other two areas. This probably reflects the greater variety of fish available as food. Of the seven families represented, the Percidae made up 55 per cent of the identified fish, the Catostomidae and Ameiuridae 17 per cent each and the Centrarchidae 13 per cent. The Hiodontidae, Cyprinidae and Esocidae were also represented. Fish over 150 mm. in length were found to be most numerous in the food items examined.

TABLE III. Stomach contents of 116 maskinonge from the Eastern Region (Quebec), 44 of which contained food.

Type of animal	Number of occurrences
Crayfish (*Cambarus virilis*)	1
Crayfish (*Cambarus* sp.)	4
Mooneye (*Hiodon tergisus*)	1
Northern sucker (*Catostomus catostomus*)	1
Common white sucker (*Catostomus commersonnii*)	1
Redhorse (*Moxostoma* sp.)	1
Bullhead (*Ameiurus* sp.)	1
Yellow perch (*Perca flavescens*)	8
Walleye (*Stizostedion vitreum*)	4
Smallmouth bass (*Micropterus dolomieu*)	2
Bass (*Micropterus* sp.)	2
Unidentified Catostomidae	2
Unidentified Cyprinidae	1
Unidentified Ameiuridae	4
Unidentified Percidae	1
Unidentified Esocidae	1
Unidentified Percomorphi	2
Unidentified fish	15
TOTAL	52

As in the other regions, *Perca flavescens* was eaten more than any other single species, forming 16 per cent of the food specimens recorded and 27 per cent of those identified. However, a single stomach was largely responsible for this predominance.

That the maskinonge will also eat large invertebrates was shown by the discovery of a 108 mm. *Cambarus virilis* freshly swallowed in one stomach. The other occurrences of *Cambarus* recorded may have been the stomach contents of the maskinonge's prey, for they were found mixed with fish remains.

Discussion and Conclusions

FOOD OF THE MASKINONGE. If most weight is placed upon the results of the large and more completely analysed sample from the Eastern Region, it may be concluded that the maskinonge is a general carnivore. The wide range of fish found in stomachs of specimens from this region and the apparent lack of significant predominance of any one species lends support to this view. In addition, several large invertebrates (*Cambarus*) were found and at least two maskinonge have been reported to have eaten muskrats. The occurrence of a pied-billed grebe in one maskinonge stomach has also been reported. Invertebrates were not eaten often, probably because of their relatively small size. Birds and mammals are seldom available to the maskinonge but are eaten when they come within range of its jaws. It would appear that the diet of the maskinonge is mainly made up of fairly large fish (over 150 mm. in length).

The high percentage of occurrence of yellow perch (*Perca flavescens*), especially in the Western and Central Regions, may have been due largely to a greater abundance of this fish than of the other species present. Similarly, the comparatively small number of other species eaten in the Western and Central Regions may merely reflect the lack of a large number of species in many of the small lakes which are common in those regions.

FOOD OF THE HYBRID MASQUINONGY × LUCIUS. Little can be concluded from the small amount of data available on the food of the hybrid found in Little Vermilion Lake in the Western Region. The only food item identified was a common white sucker (*Catostomus commersonnii*), which is also one of the common foods of the maskinonge. The pike (*Esox lucius*) has also been found to feed on this sucker (Clemens, Dymond and Bigelow, 1924; Doan, 1945) but, in some waters at least, the sucker appears to be a less common food of the pike than of the maskinonge.

Condition of Teeth During the Feeding Season

Anglers have often reported that maskinonge do not bite early in July and attribute this condition to sore mouths as the result of teeth being shed during this period. In order to investigate the possibility of this being a factor in the feeding habits of the maskinonge, the teeth of all specimens examined were checked for their general condition with respect to time of shedding and classified under the following headings:

1. *New teeth*—small, moderately firm teeth.
2. *Mature teeth*—large, strong, and firm teeth.
3. *Old or shedding teeth*— large teeth haemorrhaged at the roots and quite loose.

In all three regions nearly all the specimens examined were observed to have new, mature, and shedding teeth at the same time. It must therefore be concluded that the teeth of the maskinonge are being continuously replaced throughout the summer season and thus it is not likely that any major change in feeding habits, such as not feeding at one particular part of the summer, is attributable to the shedding of teeth. This is in agreement with the findings

of Trautman and Hubbs (1935) on the shedding of teeth in the pike. They state that "there is no evident seasonal change of consequence in the number of teeth in service, or being replaced", and, further, that the pike ". . . partakes freely of food throughout the summer: our specimens caught in July and August contained approximately as much food as those captured in cooler months". Weed (1927), on the other hand, states that an aquarium maskinonge in Chicago ". . . has been in the habit of fasting for several weeks each summer". Whether the reported low availability of maskinonge in early summer is due to feeding habits or not, it does not seem likely that the shedding of teeth is a factor involved.

GROWTH AND MATURITY

Little precise information is available on the early growth periods of the maskinonge. A study of eight small specimens taken from Lake St. Louis in Quebec (Eastern Region) by Fry, Cuerrier and Préfontaine (1941) showed a growth from a fork length of 55 mm. and a weight of 6.2 grams on June 30 to 158 mm. and 17.9 grams by October 30. Maskinonge reared in a protected area in Stoney Lake, Ontario (Elson, 1940) grew from 76-127 mm. to 203-299 mm. over a comparable period.

Schloemer (1936) made a study of the growth of the northern maskinonge (*Esox masquinongy immaculatus*) in 70 different Wisconsin lakes over an eight-year period. During this time he gathered data on 351 fish and found ". . . a rapid increase in length during the first three years, after which the growth is somewhat slower. This decrease in annual increment is correlated with a more rapid increase in weight". His calculations showed that the average maskinonge "attains the standard length of 25.7 inches corresponding to the legal size limit of 30 inches total length [to the extended tips of the tail] during the fifth summer." This last statement was based on figures from several lakes. In these individual lakes the maskinonge on the average attained their legal size between their fourth and seventh summers.

The maskinonge is a comparatively large game fish, and consequently the record sizes recognized have always attracted considerable interest. According to *Field and Stream*, the largest authentic maskinonge to be captured was a 102-pound fish netted in Lake Minocqua, Wisconsin, during spring spawn-taking operations in 1902. Unconfirmed records range up to a 112-pound maskinonge reported caught by Indians in Byng Inlet, Georgian Bay (*Rod and Gun*, 1926). Numerous catches of specimens between 40 to 60 pounds have been recorded from various places in all of the three regions studied. The largest maskinonge examined in this study was a fish of 38 lb. (17.3 kg.), measured as 52⅜ inches (1332 mm.) long to the fork of the tail. It was caught in the Lake of Two Mountains on the St. Lawrence River.

Materials and Methods

Three measurements of length were made on all specimens: (1) standard length, measured to the end of the last vertebra excluding the hypural plate; this point being determined by probing; (2) fork length, to the tip of the

shortest complete ray of the caudal fin; (3) natural tip length or total length measured along the mid-line of the body to a perpendicular drawn from the longer lobe of the tail fin, when the fin lies in a natural position. The extreme tip length (to the longer tip of the fin when squeezed to maximum extension) was not taken; not only is it difficult to compress a maskinonge's tail into this position, but anglers would often object to such mutilation of their fish. The relationships between these three measurements are shown in Table IV. An

TABLE IV. Mean fork length and conversion factors for changing fork length into standard length and natural tip ("total") length, for maskinonge of each of the five length groups in the three regions studied.

		Fork length in mm.				
		301–500	501–700	701–900	901–1100	1101–1300
Number of specimens	Western	..	2	52	20	2
	Central	1	18	36	10	2
	Eastern	1	10	98	76	28
Mean fork length in mm.	Western	..	680.0	801.9	971.9	1104.5
	Central	359	651.2	759.2	984.9	1159.0
	Eastern	426	617.8	814.3	979.9	1163.1
Conversion factor, fork length to standard length	Western	..	0.9120	0.9181	0.9219	0.9180
	Central	0.911	0.9233	0.9180	0.9311	0.8940
	Eastern	0.906	0.9224	0.9276	0.9311	0.9367
Conversion factor, fork length to "total" length	Western	..	1.0550	1.0586	1.0547	1.0550
	Central	1.097	1.0817	1.0757	1.0644	1.0545
	Eastern	1.080	1.0629	1.0606	1.0548	1.0476

approximate factor for converting fork length to extreme tip length (the more usual "total" length elsewhere) is estimated to be 1.08 for fish 500-1000 mm. long. In this paper the term "length" always means fork length, unless qualified.

After measuring a fish, its weight was recorded, and a sample of at least six scales was taken for age determination. Weight was determined to the nearest quarter pound, or where possible to the nearest ounce, using a spring balance. The balance was checked from time to time with reliable scales, and was never found to be sufficiently incorrect to alter the weights determined within the degree of accuracy recorded. Age was determined from the scales in the manner outlined by Beckman (1942). All scales were mounted in Defauré's solution (Kennedy, 1943). Some difficulty was encountered in determining the position of the later annuli in the scales of older fish. In such cases the circuli were so fine and so close together and a year's growth was so small that "cut-offs" could not be distinguished. The growth for these years had to be estimated largely on the basis of the distance between circuli.

RESULTS

AGE-LENGTH RELATIONSHIP. Plots of the individual age determinations against the corresponding fork length measurements showed a slight sexual dimorphism,

in that females seemed to be longer than males in most of the age groups. The data were therefore tabulated and tested by analysis of variance using multiple classifications (Snedecor, 1937). The data from the Western and Central Regions tended to have the sub-class numbers proportional and were treated as such. This condition was not satisfied by the eastern data, which required the additional analysis accorded to data with disproportionate sub-class numbers. The resulting interaction factor was not significant, thus making more detailed analysis unnecessary. The results of these analyses are tabulated by regions in Tables V, VI and VII.

TABLE V. Analysis of variance of length with age and sex for maskinonge from the Western Region.

Source of variation	Degrees of freedom	Sum of squares	Mean square
Age	5	225,694	45,139
Sex	1	29,595	29,595
Age-sex interaction	5	32,877	6,575
Within subclasses	44	210,597	4,786
Total	55	288,166	
For ages, F = 45139/4786 = 9.43		$P_{05} = 2.43$	$P_{01} = 3.46$
For sexes, F = 29595/4786 = 6.18		$P_{05} = 4.06$	$P_{01} = 7.24$
For interaction, F = 6575/4786 = 1.37		$P_{05} = 2.43$	$P_{01} = 3.46$

TABLE VI. Analysis of variance of length with age and sex for maskinonge from the Central Region.

Source of variation	Degrees of freedom	Sum of squares	Mean square
Age	4	194,818	48,705
Sex	1	227	227
Age-sex interaction	4	1,675	419
Within subclasses	23	68,150	2,963
TOTAL	32	264,870	
For ages, F = 48705/2963 = 16.44		$P_{05} = 2.80$	$P_{01} = 4.26$
For sexes, F = 227/2963 = 0.077		$P_{05} = 4.28$	$P_{01} = 7.88$
For interaction, F = 419/2963 = 0.141		$P_{05} = 2.80$	$P_{01} = 4.26$

From the tables it may be seen that the expected increase in length with age was found in all three regions. The difference in length between the sexes was found to be significant for the western data and highly significant for the eastern data. It would therefore seem that there is a sexual dimorphism in the length of the maskinonge, the female fish tending to grow faster and to become longer. The data from the Eastern Region represent a greater age span

(4-12 years) and show this dimorphism quite clearly. The small size of the central fish and the short range in ages involved (4-9 years) probably caused the apparent lack of significant sexual dimorphism, as any such difference would certainly show up more clearly in the larger fish. In addition, this sample was

TABLE VII. Analysis of variance of length with age and sex for maskinonge from the Eastern Region.

Source of variation	Degrees of freedom	Sum of squares	Mean square
Age	8	1,934,613	241,827
Sex	1	355,955	355,955
Age-sex interaction	8	67,327	8,416
Within subclasses	189	1,019,783	5,396
Total	206		
For ages F = 241827/5396 = 44.82		$P_{.05} = 1.98$	$P_{.01} = 2.60$
For sexes, F = 355955/5396 = 65.97		$P_{.05} = 3.89$	$P_{.01} = 6.76$
For interaction, F = 8416/5396 = 1.56		$P_{.05} = 1.98$	$P_{.01} = 2.60$

by far the smallest and contained only ten male fish. The sample from the Western Region, where a significant dimorphism was found, was larger, had a more equal division of the sexes, and was made up of older fish (8-13 years).

TABLE VIII. The relationship of fork length to age by regions.

	Eastern Region		Central Region		Western Region		*masquinongy* × *lucius* hybrid from Western Region	
Age in years	Mean length in mm.	Number in class	Mean length in mm.	Number in class	Mean length in mm.	Number in class	Mean length in mm.	Number in class
2	467	2	472	2	..	..	..	..
3	587	3	567	3	..	..	..	..
4	692	8	714	18	..	..	..	..
5	798	17	725	15	..	..	..	..
6	824	46	772	9	673	1	..	..
7	876	51	778	8	769	5	..	..
8	956	41	938	4	745	11	..	..
9	1056	21	958	5	838	21	916	1
10	1137	12	1071	4	812	7	973	1
11	1125	7	..	..	890	13	994	1
12	1093	7	..	..	897	5	966	2
13	..	..	..	..	968	7	..	..
14	..	..	..	..	894	1	..	..

Thus the dimorphism in length would appear to be relatively small or even non-existent in the younger age groups, becoming more pronounced as the fish grows older.

It should perhaps be noted that since the amount of the variation in length increases with increase in mean length, the use of the logarithms of the lengths rather than the actual lengths might provide a more satisfactory analysis of this type of data.

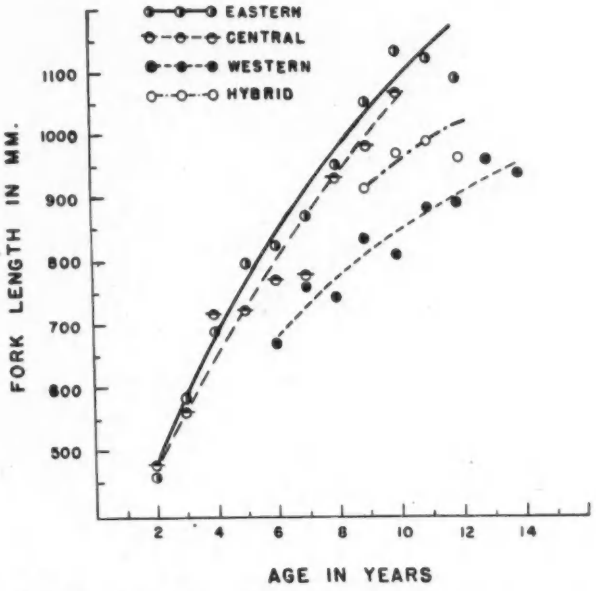

FIGURE 2. The relationship of fork length to age for the maskinonge from each of the three regions studied.

In order to compare the age-length relationships of the different regions, the sexes were combined. This step seemed justified since little or no sexual dimorphism in length was noted in the younger fish, and in the older age groups where dimorphism was evident, females were more abundant in all three regions. The data were grouped by ages and the mean length calculated for each year-class (Table VIII). In Figure 2 a curve for each region has been drawn by inspection.

1. Although the maskinonge from the Eastern and Central Regions did not seem to differ appreciably in rate of growth, those from the Western Region had considerably slower growth. A more detailed statistical analysis for possible further differences is not warranted because of the assumptions made in grouping the data and the lack of overlap of the heavily sampled age groups from the Western and Central Regions.

2. The specimens of the *masquinongy* × *lucius* hybrid had faster growth than did the specimens of maskinonge from the same region, but not as fast growth as that of the maskinonge from the other two regions.

3. The age-length relationships of the maskinonge from each region all have a curvilinear trend, the growth in length per year decreasing with age.

TABLE IX. Analysis of variance of weight with age and sex for maskinonge from the Western Region.

Source of variation	Degrees of freedom	Sum of squares	Mean square
Age	5	393.60	78.72
Sex	1	58.24	58.24
Age-sex interaction	5	98.51	19.70
Within subclasses	42	546.07	13.00
TOTAL	53	1,096.42	
For ages, F = 78.72/13.00 = 6.06		P_{05} = 2.32	P_{01} = 3.26
For sexes, F = 58.24/13.00 = 4.48		P_{05} = 4.07	P_{01} = 7.27
For interaction, F = 19.70/13.00 = 1.51		P_{05} = 2.32	P_{01} = 3.26

TABLE X. Analysis of variance of weight with age and sex for maskinonge from the Central Region.

Source of variation	Degrees of freedom	Sum of squares	Mean square
Age	4	361.27	90.32
Sex	1	1.12	1.12
Age-sex interaction	4	0.40	0.10
Within subclasses	20	86.16	4.10
Total	29	448.95	
For ages, F = 90.32/4.10 = 22.03		P_{05} = 2.87	P_{01} = 4.43
For sexes, F = 1.12/4.10 = 0.27		P_{05} = 4.35	P_{01} = 8.10
For interaction, F = 0.10/4.10 = 0.02		P_{05} = 2.87	P_{01} = 4.43

AGE-WEIGHT RELATIONSHIP. Tabulations of the individual age and weight determinations showed the possibility of a sexual dimorphism, females tending to be heavier than males of the corresponding age. The data for each region were tested by analysis of variance in the same way as was done for lengths (Tables IX-XI). A highly significant variation (increase) in weight with age was found in all three sets of data. The variation in weight with sex was found to be significant in the Western Region and highly significant in the Eastern Region. The age-sex interaction was significant only in the Eastern Region.

Such an interaction is best interpreted by means of further computation (Snedecor, 1937, p. 291) but because the age and sex variations were so much larger than the interaction, the simple analysis should be very little distorted.

Thus, where the older age groups are present (Western and Eastern Regions), a sexual dimorphism in weight was found—that is, a significant variation of weight with sex. This in turn would indicate that, as in the case of the age-length relationship, the dimorphism either develops at a later age or, more likely, increases with age. Support for this view comes from the small but significant age-sex interaction found in the Eastern Region—that is, a tendency for the difference in weight of the sexes to increase with age. By contrast, the fish from the Central Region were mainly from the younger age

TABLE XI. Analysis of variance of weight with age and sex for maskinonge from the Eastern Region.

Source of variation	Degrees of freedom	Sum of squares	Mean square
Age	8	3,833.30	479.16
Sex	1	843.53	843.53
Age-sex interaction	8	299.45	37.43
Within subclasses	196	2,724.14	13.90
TOTAL	213		
For ages, $F = 479.16/13.90 = 34.46$		$P_{05} = 1.98$	$P_{01} = 2.60$
For sexes, $F = 843.53/13.90 = 60.69$		$P_{05} = 3.89$	$P_{01} = 6.76$
For interaction, $F = 37.43/13.90 = 2.69$		$P_{05} = 1.98$	$P_{01} = 2.60$

groups (4-6 years) and showed a spread of only six years, consequently they were too young for the sexual dimorphism in weight to be demonstrable. The Western fish also showed a spread of only six years, but these fish were old enough to show a significant sexual dimorphism in weight; however, the age spread was too small to show any change in its magnitude. In the Eastern Region samples were spread over nine years of age (4-12 years), making possible a demonstration of not only sexual difference in weight, but also the fact that the difference increases with age.

For a comparison of the age-weight relationships of the three regions, the sexes were lumped. Since little or no sexual dimorphism was found in the younger age-groups and since females were more abundant in the older age-groups of all regions, it was considered that lumping the sexes would not invalidate the results of a comparison by regions. The data were then grouped by ages and the mean weight calculated for each year-class (Table XII). Figure 3 shows the age-weight relationship graphically; the trend of each set of data is shown by a curve drawn by inspection.

1. The maskinonge from the Western Region were considerably lighter than those of comparable ages from the other two regions. The Central fish were, in turn, lighter than the Eastern fish, but only slightly so, as in the case of the age-length relationship.

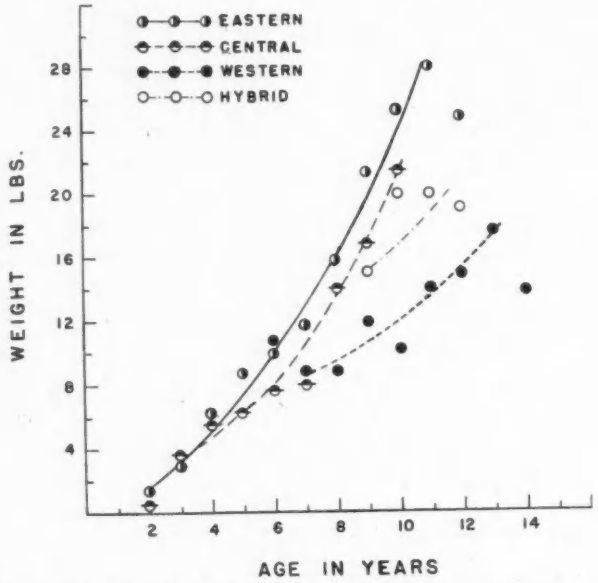

FIGURE 3. The relationship of weight to age for the maskinonge from each of the three regions studied.

2. The *masquinongy* × *lucius* hybrids from the Western Region were distinctly heavier than the maskinonge from the same region but not as heavy as the maskinonge from the other two regions.

3. The age-weight relationships of the maskinonge from each region showed a curvilinear trend, the annual increment in weight increasing with age.

LENGTH-WEIGHT RELATIONSHIP. No sexual dimorphism was indicated when the individual weight determinations were plotted against the corresponding fork-length measurements. The sexes were therefore lumped, the data grouped in intervals of 100 mm. and the average weight was calculated for each interval (Table XIII). A graph of the logarithm of average weight against the logarithm of fork length is shown in Figure 4. From this figure it may be noted that:

1. No significant difference was evident in the length-weight relationship of maskinonge from the Eastern and Central Regions. However, in every length interval, maskinonge from the Western Region had a greater weight than did the maskinonge from the other two regions. The probability of all the points representing the Western Region lying above those of the Eastern and Central

Regions is roughly one in thirty-two. This does not take into account the numbers of fish involved in each length class, but it does permit considerable confidence in the difference between the western fish and those of the other two regions. The Western fish tend to be heavier for their length than the others.

TABLE XII. The relationship of weight to age by regions.

	Eastern Region		Central Region		Western Region		*masquinongy* × *lucius* hybrid from Western Region	
Age in years	Mean wt. in lb.	No. in class	Mean wt. in lb.	No. in class	Mean wt. in lb.	No. in class	Mean wt. in lb.	No. in class
2	1.4	2	0.5	2	..	..	..	..
3	3.2	3	2.6	3	..	..	..	..
4	5.8	8	5.8	17	..	..	..	..
5	8.7	17	6.2	12	..	..	..	..
6	9.9	47	7.6	7	10.5	1	..	..
7	11.8	54	8.0	8	8.9	6	..	..
8	15.8	43	14.0	4	8.9	12	..	..
9	21.3	19	16.8	5	11.9	19	15.0	1
10	25.3	17	21.4	4	10.1	7	20.0	1
11	28.0	9	..	..	14.0	11	20.0	1
12	24.9	6	..	..	14.9	4	19.1	2
13	..	..	..	..	17.6	8	..	..
14	..	..	..	..	13.8	1	..	..

TABLE XIII. The relationship of weight to fork length by regions.

	Eastern Region		Central Region		Western Region		*masquinongy* × *lucius* hybrid from Western Region	
Length in mm.	Mean wt. in lb.	No. in class	Mean wt. in lb.	No. in class	Mean wt. in lb.	No. in class	Mean wt. in lb.	No. in class
300– 399	..	..	0.6	1	..	..	..	..
400– 499	1.0	1	..	..	..	..	..	..
500– 599	2.5	4	2.6	4	..	..	..	..
600– 699	4.6	6	4.9	13	8.6	2	..	..
700– 799	7.3	42	6.6	25	8.2	27	..	..
800– 899	10.6	58	9.3	7	12.4	24	..	..
900– 999	14.8	47	16.1	7	16.7	14	18.9	6
1000–1099	20.4	27	17.5	3	22.4	5	..	..
1100–1199	20.2	20	26.1	2	28.1	2	..	..
1200–1299	31.1	8	..	..	..	..	..	..

2. The length-weight relationship of the *masquinongy* × *lucius* hybrid did not appear to differ significantly from that of the maskinonge.

3. In all three regions, the gain in weight per unit increase in length became greater as the fish got larger. This relationship is indicated by the linear trend

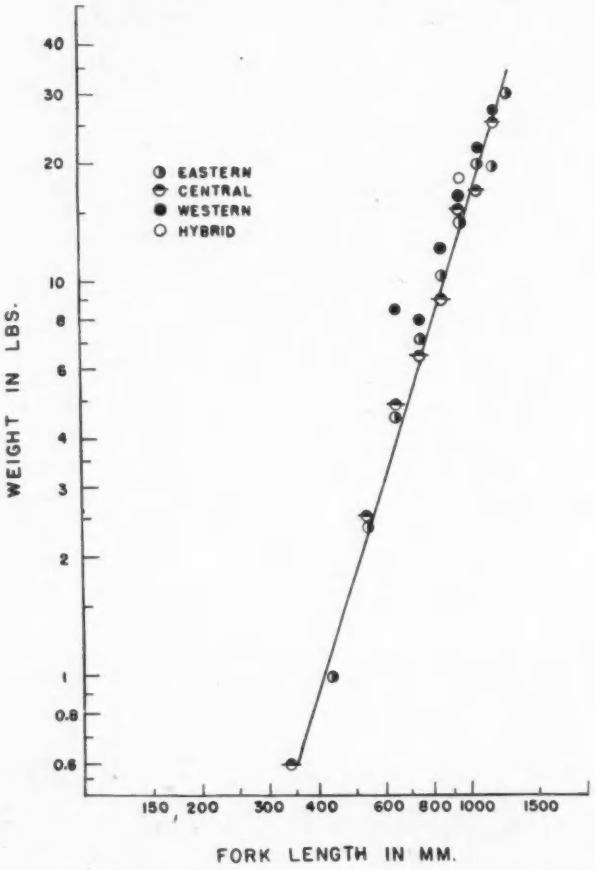

FIGURE 4. The relationship of weight to fork length, plotted logarithmically, for the maskinonge from each of the three regions studied.

in the logarithmic plot (Figure 3). A line was drawn by inspection to fit the data from the Eastern and Central Regions. Its equation was calculated and found to be:

$$\log W = 3.259 \log L - 8.540$$

The data for the western fish all lie to the left of this line but the range sampled is insufficient and the differences too small to permit comparison of

its linear trend with that of the data from the other two regions. This tendency for western fish to be heavier than those of similar length from the other two regions may be related to the fact that the western fish are shorter in length than those of similar age from the other two regions. Indeed, on the basis of age, the western fish are lighter than those from the other two regions. The difference in length would therefore appear to be the most marked of the differences between regions.

MATURITY

From records made of the sex and state of maturity of each specimen it was possible to extract an idea of when the maskinonge matures. The youngest and smallest mature specimens, and the oldest and largest immature ones from each region were noted. These data are summarized in Table XIV. With one

TABLE XIV. Range of stage of visible differentiation of gonads by regions.

Region	Length of shortest female	Age of youngest female	Length of shortest male	Age of youngest male	Length of longest immature	Age of oldest immature
	mm.	*years*	*mm.*	*years*	*mm.*	*years*
Western	687	7	673	6	..	..
Central	641	5	680	4	556	3
Eastern	591	3	587	4	726	4

exception, the same fish fitted the corresponding characteristic mentioned above for both age and length classifications. The exception occurred in the Western Region, where the shortest female was eight years old, while the youngest female was 719 mm. long.

From the table it may be seen that the most likely indication of the time of maturity would come from the data from the Central and Eastern Regions. The fish examined in the Western Region were all rather large, no immature specimens were examined, and the youngest mature ones were too large to indicate the time of maturity. The data from the other two regions showed that the gonads of the maskinonge probably differentiate visibly (that is, begin to mature) between the ages of three and five years, and approximately between the lengths of 575 and 750 mm. There appeared to be little or no difference in the time of maturity of the two sexes. It is to be noted that these values were based upon the time when sexual differentiation was noted in the gonad and that such fish may not have spawned that year. Thus the time of maturity may be taken as sometime between the ages listed and a year later. If this hypothesis holds, these data correspond closely to those for the age of attainment of legal size by maskinonge in Wisconsin lakes as determined by Schloemer (1936).

None of the gonads of the specimens of the *masquinongy* × *lucius* hybrid had matured, nor could their sex be determined by gross examination, although these fish were all large and nine to twelve years old. It would therefore seem that this form is sterile throughout life.

Discussion and Conclusions

VARIATION IN RATE OF GROWTH WITHIN REGIONS. The large variation shown in rate of growth is not unexpected in data of this type. In every region specimens were taken from more than one body of water and thus from more than one population, and the same rate of growth would not be expected for each of several populations. It is understandable then that the variation was greater in the Western and Central Regions, where the maskinonge were taken from numerous small, partially isolated lakes and rivers, than in the Eastern Region where most of the specimens came from the St. Lawrence River.

TABLE XV. Contribution of the sexes to the various length groups for each of the three regions studied.

Region	Sex	Fork length in mm. 301–500	501–700	701–900	901–1100	1101–1300	Total
Western	Male	..	1	18	1	..	20
	Female	..	1	32	10	1	44
	Undetermined	..	..	4	7	1	12
	Total	..	2	54	18	2	76
Central	Male		3	6	1	..	10
	Female	..	4	16	3	1	24
	Undetermined	1	11	15	5	1	33
	Total	1	18	37	9	2	67
Eastern	Male	..	4	64	29	..	97
	Female	..	3	36	47	27	113
	Undetermined	1	3	1		1	6
	Total	1	10	101	76	28	216

TABLE XVI. Contribution of the sexes to the various age groups for each of the three regions studied.

Region	Sex	Age in years 2	3	4	5	6	7	8	9	10	11	12	13	14	?	Total
Western	Male	..	..	..	..	1	..	3	5	4	4	2	2	..	..	21
	Female	..	..	..	..	..	4	10	8	4	7	2	5	1	2	43
	Undetermined	..	..	..	..	..	1	..	5	..	2	..	1	..	3	12
	Total	..	..	..	..	1	5	13	18	8	13	4	8	1	5	76
Central	Male	..	..	2	4	2	1	..	1	..	..	..	..	..	..	10
	Female	..	..	8	5	4	2	2	1	2	..	..	..	..	..	24
	Undetermined	2	3	8	5	3	5	2	3	2	..	..	..	..	..	33
	Total	2	3	18	14	9	8	4	5	4	..	..	..	..	..	67
Eastern	Male	..	..	5	12	22	26	21	8	..	2	2	..	..	1	99
	Female	..	2	2	5	23	24	20	12	12	5	5	..	..	1	111
	Undetermined	2	1	1	..	..	1	..	1	..	..	..	..	..	..	6
	Total	2	3	8	17	45	51	41	21	12	7	7	..	..	2	216

SEXUAL DIMORPHISM IN RATE OF GROWTH. It has been noted in this (Table XV) and other studies that the largest maskinonge taken are almost invariably females; that is, females attain a greater size than do males. This could be a result of a greater longevity on the part of the female, a faster rate of growth, or both. From Table XVI it may be seen that, on the average, there is a slight tendency for females to be older than males. This difference is, however, very small. On the other hand, a statistically significant sexual dimorphism was found in both the length and the weight of the maskinonge examined during this study, the females tending to be larger at any given age. This difference was most pronounced in the older age groups. It would therefore seem that the largest maskinonge are females mainly because of the faster rate of growth of the female, but a slight tendency for the females to outlive the males may also be a factor.

AGE-LENGTH RELATIONSHIP. The specimens from the Eastern Region seemed to have a significantly higher rate of increase in length than did the specimens from the Western Region. Any error that might be brought in by the lumping of sexes would tend to make the Western fish, with females outnumbering males two to one, longer proportionately than the Eastern ones, whose sexes are more equally represented, thus minimizing the difference. Evidently the difference in growth rate is real.

The age-length relationship for maskinonge from the Central Region did not appear to differ significantly from that found in the Eastern Region. The higher proportion of females in this region (outnumbering the males three to one) would tend to minimize any difference which might have existed since their higher rate of growth would tend to increase the rate for the combined sexes. It is not likely however, that it could have had so great an effect as to eliminate a significant difference. A detailed statistical analysis of this problem was impractical because of the assumptions made in grouping the data and the small size of the central sample. Thus the rate of growth of the maskinonge in the Central Region has not been shown to differ from that in the Eastern Region.

From the results of this study it would appear that maskinonge in the Eastern and Central Regions attain their legal size of 30 inches fork length during their fifth summer. Schloemer (1936, 1938) found that 351 maskinonge from seven Wisconsin lakes attained a somewhat smaller legal size—30 inches extreme tip length, or about 28 inches fork length—between their fourth and seventh summers; on the average the legal size was attained during the fifth summer. Lake of the Woods fish of this size were in their seventh summer. The average lengths for the various age-groups in these two studies are quite comparable also, although there seems to be a slight tendency for the older Canadian fish to be longer than fish in the corresponding age-groups in Schloemer's study. Williams (1948) found the maskinonge in Michigan to attain the extreme tip length of 32 inches (813 mm.) at the age of five years. This also corresponds with the results of the present study wherein the eastern and central fish

attained a comparable size (fork length of 752 mm.) towards the end of the fifth summer or early in the sixth summer. Maskinonge of similar size in the Western Region were in their eighth summer. Moore (1925) found a comparable rate of growth for 20 specimens of Chautaqua maskinonge (*Esox ohioensis* Kirtland), although these fish seemed to show a slightly higher rate of growth at more advanced ages. These latter age groups in Moore's study consisted of only one fish in most cases so the small difference encountered could hardly be significant. The average lengths of age groups in the two studies show relatively close agreement over the range which is best sampled.

AGE-WEIGHT RELATIONSHIP. Maskinonge from the Western Region weighed considerably less than those of comparable ages from the other two regions. Here again, any error introduced by lumping the sexes after females had been shown to be slightly heavier than males would tend to make the Western fish proportionately heavier than the Eastern fish and thus minimize the difference in weight between them. (The male to female ratio was one to two in the Western Region and one to one in the Eastern Region.)

The Central Region fish were also lighter in weight than fish of comparable ages in the Eastern Region. However, this difference was slight and some overlap of means occurred. In spite of the fact that the sexual dimorphism in weight would tend to minimize this difference (the male to female ratio was one to three for the Central data and one to one for the Eastern data), the difference would not appear to be significant when the range of variation in the individual weights is considered. The small size of the Central Region sample and the assumptions made in grouping the data precluded a detailed statistical analysis of this problem as its results would lack biological significance.

LENGTH-WEIGHT RELATIONSHIP. None of the length-weight relationships showed any indication of sexual dismorphism in the maskinonge from the three regions studied. No significant difference was noted in the length-weight plots of fish from the Central and Eastern Regions, but the western fish were consistently heavier at any given length group than were those from the other two regions. This relationship may in turn be related to the shorter average length of the western maskinonge which was found in all age groups, since the western fish weigh less than those of comparable age from the other two regions. The data showed a linear trend when plotted logarithmically, with a slope of 3.259. The correspondence between length and weight was similar among maskinonge from Lake St. Clair (Krumholz, 1949) and in Michigan waters (Williams, 1948).

MATURITY. The gonads of the maskinonge become visibly differentiated (that is, begin to mature) between the ages of three and five years and the fork lengths of 575 and 750 mm. Most of these fish would not spawn for the first time until the following year and thus the maskinonge probably matures when between three and six years of age, or approximately 575 to 800 mm. fork length. This agrees closely with Schloemer's (1938) study in Wisconsin where the maskinonge were found to mature between the ages of three and six years.

MASQUINONGY × LUCIUS HYBRID. The length-weight relationship of the *masquinongy* × *lucius* hybrid did not appear to differ significantly from that of the maskinonge. The hybrid had a higher rate of growth both in length and weight, than did specimens of the maskinonge from the same region, and appeared to be infertile. This increased rate of growth might be attributed to hybrid vigour. A similar condition was found by Hubbs and Hubbs (1933) for hybrid sunfish.

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LITERATURE CITED

ANONYMOUS. *Rod and Gun in Canada*, **27**, 573, 1926.

ANDERSON, L. R. Unusual items in the diet of the northern muskellunge (*Esox masquinongy immaculatus*). *Copeia*, **1948**, 63, 1948.

BECKMAN, W. C. Annulus formation on the scales of certain Michigan game fish. *Papers Michigan Acad. Sci. Arts Letters*, **28**, 281-312, 1942.

CAMERON, G. S. An unusual maskinonge from Little Vermilion Lake, Ont. *Can. Jour. Res.*, D, **26**, 223-229, 1948.

CLEMENS, W. A., DYMOND, J. R. AND N. K. BIGELOW, Food Studies of Lake Nipigon fishes. *Univ. Toronto Stud., Biol. Ser.*, No. 25 (*Pub. Ontario Fish. Res. Lab.*, No. 25), 103-165, 1924.

DOAN, K. H. The control of the jackfish, *Esox lucius*, in Heming lake, Manitoba, in relation to the parasitization of whitefish by the tapeworm *Triaenophorus crassus*. *Fisheries Res. Bd. Canada, MS. Rept. of Cent. Fish. Res. Sta.*, No. 11, 1-40, 1945.

DYMOND, J. R. A list of the freshwater fishes of Canada east of the Rocky Mountains. *Roy. Ontario Mus. Zool., Misc. Publ.*, No. 1, 1-36, 1947.

EDDY, S., AND T. SURBER. Northern fishes. 252 pp. Minneapolis: Univ. Minnesota Press, 1943.

ELSON, P. F. Rearing maskinonge in a protected area. *Monthly Bull. Ont. Dept. Game Fish.*, **1940**, 421-429, 1940.

FRY, F., J. P. CUERRIER AND G. PREFONTAINE. Première croissance du maskinonge dans le lac Saint-Louis en 1941. *Rapport de la Station Biologique de Montréal et de la Station Biologique du Parc des Laurentides pour l'Année 1941*, 171-175, 1941.

HUBBS, C. L., AND L. C. HUBBS. The increased growth, predominant maleness and apparent infertility of hybrid sunfishes. *Pap. Michigan Acad. Sci. Arts Letters*, **17**, 613-641, 1933.

KENNEDY, W. A. The whitefish, *Coregonus clupeaformis* (Mitchell), of Lake Opeongo, Algonquin Park, Ont. *Univ. Toronto Stud.*, No. 51 (*Pub. Ontario Fish. Res. Lab.*, No. 62), 23-66, 1943.

KRUMHOLZ, L. A. Length-weight relationship, *Esox m. masquinongy* in Lake St. Clair. *Trans. Amer. Fish. Soc.*, **77**, 42-48, 1949.

MOORE, EMMELINE. Problems in fresh water fisheries. Part II. Culture of the maskinonge (muskellunge). *Ann. Rept. New York State Cons. Comm. 1925*, 131-137, 1925.

SCHLOEMER, C. L. The growth of the muskellunge, *Esox masquinongy immaculatus* (Garrard), in various lakes and drainage areas of northern Wisconsin. *Copeia*, **1936**, 185-192, 1936.

SCHLOEMER, C. L. A second report on the growth of the muskellunge *Esox masquinongy immaculatus* (Garrard) in Wisconsin waters. *Trans. Wisc. Acad. Sci. Arts Letters*, **31**, 507-512, 1938.

SNEDECOR, G. W. Statistical Methods. 485 pp. Iowa State College Press, 1937.

TRAUTMAN, M. B. AND C. L. HUBBS. When do pike shed their teeth? *Trans. Amer. Fish. Soc.*, **65**, 261-266, 1935.

WEED, A. C. Pike, pickerel and muskalonge. *Field Mus. Nat. Hist. Zoology Leaflet*, No. 9. Chicago, 1927.

WILLIAMS, J. F. The muskellunge in Michigan. *Michigan Cons.*, **17**, 10, 10-11, 15, 1948.

The Relation of Digging Frequency to Productivity of the Butter Clam, Saxidomus giganteus Deshayes

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ABSTRACT

The effect of frequency of digging butter clams, *Saxidomus giganteus*, on production was tested by the Latin square method in five different localities of southern British Columbia. Digging once in 7 years appeared somewhat less productive than digging every ½, 1, 2 or 3 years. Among the latter four frequencies there were no detectable differences.

PROCEDURE

This experiment was designed to test which of five given frequencies of digging would give, over a period of years, the greatest yield of butter clams from British Columbia clam beaches.

The butter clam is the most abundant of the species used commercially in British Columbia. Its habitat is in the lower levels of the intertidal area, and as it grows it burrows to a maximum depth of twelve inches in soils composed of various proportions of sand, mud, gravel and shell. Commercial digging takes place largely during the winter months.

The experiment was designed after R. A. Fisher's Latin square method of field plot experimentation (Fisher and Wishart, 1930). Details of the method are not given here as both practical and theoretical considerations may be found in the special literature on Latin square plot experiments and in recognized statistical texts. Randomization was carried out along the lines considered suitable by Yates (1933).

Plots were subjected to the following five digging frequencies or "treatments".

(1) Twice per year
(2) Once per year
(3) Every second year
(4) Every third year
(5) At the end of the experiment

Square blocks were marked out on five beaches, each located near the center of a major clam-producing area along the east coast of Vancouver Island. Excepting the Departure Bay block, which was fifty feet square, the dimensions

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Knapp Island

14_3	9_5	21_4	10_1	14_2	68
24_1	17_4	24_2	18_3	18_5	101
5_5	17_2	19_1	24_4	15_3	80
19_2	23_1	14_3	12_5	20_4	88
22_4	18_3	12_5	21_2	16_1	89
84	84	90	85	83	

Departure Bay

22_3	42_1	31_5	93_2	79_4	267
9_5	25_4	41_2	55_1	120_3	250
10_4	20_2	39_1	59_3	43_5	171
4_1	4_3	17_4	57_5	54_2	136
3_2	2_5	19_3	62_4	38_1	124
48	93	147	326	334	

Dunsmuir Island

9_3	15_1	1_5	16_2	6_4	47
6_5	4_3	7_2	4_4	13_1	34
9_4	8_2	6_1	4_3	4_5	31
9_1	10_4	12_3	5_5	8_2	44
5_2	4_5	10_4	9_1	5_3	33
38	41	36	38	36	

Seal Island

44_4	50_1	79_2	179_5	61_3	413
46_1	34_2	58_3	61_4	41_5	240
75_5	43_3	49_4	30_2	44_1	241
28_3	51_4	44_5	39_1	45_2	207
40_2	28_5	31_1	40_3	50_4	189
233	206	261	349	241	

Echo Bay

25_1	35_4	17_3	25_5	45_2	147
55_2	28_5	31_4	35_3	32_1	181
36_5	33_3	44_2	39_1	47_4	199
27_4	40_1	18_5	36_2	45_3	166
33_3	29_2	31_1	29_4	32_5	154
176	165	141	164	201	

FIGURE 1. Latin squares giving the seven-year plot yields of butter clams and the totals for rows and columns. Figures are in pounds per 25 square-foot plot, except for Departure Bay where the plot is 100 square feet. Subscript figures are the treatment numbers. The offshore side of each square is at the top.

of the experimental areas were 25 feet by 25 feet, giving 25 five-foot-square plots. The smaller blocks were probably more efficient as their compactness relieved the experiment of excessive fertility gradients.

The plots were dug during the normal commercial season as far as possible and the commercial methods of digging were followed as closely as the nature of the experiment allowed. In digging, other species were neglected (they did not occur in great abundance in any of the plots) and butter clams under the legal length of two and one-half inches were carefully returned to the plot of origin. The clams from each plot were sacked as they were dug and weighed whole (shell and meat) to the nearest ounce after draining for a period of eight to twelve hours.

TABLE I. Analysis of variance of yields from the experimental areas.

Experiment	Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	"F" Value	Significance
Knapp Island	Rows	4	122.96	30.74		
	Columns	4	9.16	2.29		
	Treatment	4	281.36	70.34	4.46	Significant (P = 0.019)
	Error	12	189.48	15.79		
	Total	24	602.96	25.12		
Dunsmuir Island	Rows	4	41.36	10.34		
	Columns	4	3.36	0.84		
	Treatment	4	102.16	25.54	1.67	Nil
	Error	12	183.28	15.27		
	Total	24	303.16	12.63		
Departure Bay[a]	Rows	4	213.62	53.40		
	Columns	4	881.96	220.49		
	Treatment	4	51.02	12.75	0.79	Nil
	Error	12	193.80	16.15		
	Total	24	1340.40	55.85		
Seal Island	Rows	4	6400.00	1600.00		
	Colums	4	2381.60	595.40		
	Treatment	4	3175.60	793.90	1.06	Nil
	Error	12	8978.80	748.23		
	Total	24	20,936.00	872.33		
Echo Bay	Rows	4	352.24	88.06		
	Columns	4	379.44	94.86		
	Treatment	4	507.84	126.96	2.33	Nil
	Error	12	653.12	54.43		
	Total	24	1,892.64	78.86		

[a]Adjusted to compare with smaller plots by dividing each yield by 4.

The experiment was concluded in 1945 after a period of seven years.

The weights of the clams derived from all the diggings of each individual plot were summed and rounded to the nearest whole pound. The weights represent the total produce of butter clams from each of the plots for the seven years of the experiment (Figure 1). These results were treated by the analysis of variance (Table I), Fisher (1925, 1942), Snedecor (1928). In Table II is given the mean yields and the associated standard errors. Snedecor's "F" adaptation of Fisher's "z" table was used for testing significance.

DISCUSSION

Perusal of Table II shows that the standard error of the mean yields from the plots is invariably high, which reflects a large residual variability in yield even after "fertility" gradients on the sites have been eliminated as far as possible by the Latin square arrangement.

The sums of squares for "rows" tend to be moderately large (Figure 1). Since rows run parallel to the shore, variation in depth of water may be pre-

TABLE II. Mean yield of butter clams per 25 square feet of bottom area for different digging intervals.

Experiment	Interval (see text for description)					Mean yield per plot	Standard error
	1	2	3	4	5		
Knapp Island	18.4	19.0	15.8	20.8	11.2	17.04	8.89
Departure Bay	8.9	10.5	11.2	9.6	7.1	9.46	8.99
Dunsmuir Island	10.4	8.8	6.8	7.8	4.0	7.56	8.74
Seal Island	42.4	45.6	46.0	51.0	73.4	51.68	61.16
Echo Bay	33.4	41.8	32.6	33.8	27.8	33.88	16.49

sumed to be partly responsible for this effect. The "columns" sums of squares vary from large to inconsiderable. At Departure Bay there was a marked productivity gradient, increasing from east to west across the site (left to right in Figure 1).

Considering now the effects of the different digging "treatments", only at Knapp Island was there a significant "F" for treatment as compared with error. However when the F-values for all experiments are combined in assessing significance, as is done in Table III using the χ^2 method (Fisher, 1950, sect. 21.1), the combined probability for treatment effects is $P = 0.06$, which justifies 94 per cent confidence that one or more of the treatments did differ from the others in its effect upon total yield of clams. This result does not in itself indicate which treatments are good or bad; each site might differ in respect to which treatment is most favourable. However, at the three sites which contribute appreciably to χ^2 in Table III, treatment 5 (only one digging in 7 years) is consistently the worst, and it is obvious that this treatment is principally responsible for the observed effect. The same thing is shown in Table IV, where

the yield of each treatment is computed as a percentage of the total yield for the site. The average for treatment 5 is 16.8 per cent, which is well below the others. Seal Island was exceptional in that treatment 5 had the highest mean yield; this was the result of a single very heavily populated plot (7 lbs. per square foot; see Figure 1). Treatments 1 to 4 exhibit only small differences, although No. 2 (yearly digging) did have the best total.

Digging at seven-year intervals therefore seems definitely inferior to the others, from the stand-point of total yield, and there is little to choose among intervals of ½, 1, 2 and 3 years.

TABLE III. Combined test of significance of treatment effects on all five plots.

Site	F	P	$-\log_e P$	χ^2	D.F.
Knapp Island	4.46	0.019	3.97	7.94	2
Dunsmuir Island	1.67	0.220	1.51	3.02	2
Departure Bay	0.79	0.68	0.39	0.78	2
Seal Island	1.06	0.48	0.73	1.46	2
Echo Bay	2.33	0.113	2.18	4.36	2

Total $\chi^2 = 17.56$; D.F. = 10; P = 0.04.

TABLE IV. Average yield per plot expressed as a percentage of the total yield for each site.

	Treatment				
	1	2	3	4	5
Knapp Island	21.6	22.3	18.6	24.4	13.1
Dunsmuir Island	27.5	23.3	18.0	20.6	10.6
Departure Bay	18.8	22.2	23.7	20.3	16.0
Seal Island	16.4	17.6	17.8	19.7	28.5
Echo Bay	19.7	24.7	19.2	20.0	16.4
Mean	20.7	22.0	19.5	21.0	16.8

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LITERATURE CITED

FISHER, R. A. Statistical methods for research workers. Edinburgh, 1950.

FISHER, R. A. The design of experiments. Edinburgh, 1942.

FISHER, R. A. AND J. WISHART. The arrangement of field experiments and the statistical reduction of the results. *Imperial Bureau of Soil Science, Technical Comm.*, No. 10, 1930.

SNEDECOR, G. W. Statistical Methods. Ames, Iowa, 1938.

YATES, F. The formation of Latin squares for use in field experiments. *Emp. Jour. Exper. Agric.*, **1**, 235, 1933.

Notes on Dispersion and Growth of some British Columbia Bottom Fishes

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ABSTRACT

Recoveries of tags have provided limited information on the dispersion of several British Columbia groundfishes. *Platichthys stellatus* and *Hippoglossoides elassodon* were recovered mostly within 1 to 3 years and have moved less than 5 miles; however, one *P. stellatus* shifted position by 60 miles in 5½ years. A very few recoveries of *Psettichthys melanostictus* tags suggest that movements up to 25 miles may be not uncommon for this species. *Anoplopoma fimbria* recoveries were nearly all within 10 miles of release but tagging was done under conditions favouring local recovery. During a year's freedom the average growth of a male *P. stellatus* varies from 35 mm. at 350 mm. initial size to 10 mm. at 520 mm.

At the start of the investigation of the British Columbia trawl fishery in 1943 very little was known of the life history of each species of bottom fish, or the role which it played in the general fishery. Accordingly the investigation was begun on a rather broad scale and some thirty species were handled. As the investigation proceeded it became necessary to discontinue observations on some of these, and by 1945 the number of species being studied had been reduced to about ten. In the meantime some tagging work had been carried out on most of the species later rejected, and for several of these, recoveries have been received. It appears unlikely that any additional recoveries will be made now, so the data from these experiments have been analysed and the findings concerning movement and growth of some species are reported here.

In addition to the number of tags used and the intensity and location of the fishery, the number of tagged fish recaptured depends upon:

(1) the ability of the species to withstand handling and tagging; and

(2) the liability of the tagged fish to capture or retention, as influenced by

- (*a*) the commercial value of the species,
- (*b*) association with species sought commercially,
- (*c*) their size, and
- (*d*) vulnerability to capture as a result of carrying a tag.

Usually more than one of these factors operated at the same time.

Tagging, using the Petersen type of tag, was carried out in many areas of British Columbia. No recoveries resulted from the taggings on the following species.

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REX SOLE (*Glyptocephalus zachirus*). Ninety tagged, 30-48 cm. long.
DOVER SOLE (*Microstomus pacificus*). Forty-five tagged, 27-41 cm. long.
LONG-JAW FLOUNDER (*Atheresthes stomias*). Fifteen tagged, 28-62 cm. long.
CURL-FIN SOLE (*Pleuronichthys decurrens*). Eleven tagged, 23-32 cm. long.
SLENDER SOLE (*Lyopsetta exilis*). Five tagged, 26-31 cm. long.
MOTTLED SAND DAB (*Citharichthys sordidus*). Four tagged, 22-29 cm. long.
ROCKFISH (*Sebastodes*). Fifteen tagged, 27-55 cm. long.

TABLE I. Number and range in size of fish tagged, number of recoveries, and the minimum distance travelled by each before recovery.

		Starry flounder	Sand sole	Flat-head sole	Black-cod	Gray cod
Number tagged		275	91	219	82	39
Range in size (cm.)		25–69	25–54	22–42	37–64	42–72
Number recovered		27	5	7	15	2
Days out	1– 100	..	2	3	11	2
	101– 200	2	..	..	3	..
	201– 300	..	..	1	1	..
	301– 400	15	2	..	..	..
	401– 500	5	..	1	..	..
	501– 600	2	..	..	..	..
	601– 700	1	1	..	..	..
	701– 800	1	..	..	..	..
	801– 900	..	..	1	..	..
	901–1000	..	..	..	..	..
	1001–1100	..	..	..	..	..
	1101–1200	..	..	1	..	..
	2001–2100	1	..	..	..	..
Miles travelled	0– 5	21	1	6	11	..
	6–10	..	1	..	3	1
	11–15	1	..	..	..	..
	16–20	..	..	..	..	..
	21–25	..	2	..	1	..
	56–60	1	..	..	..	..

Of these, it was observed that the dover sole and particularly the rex sole reacted badly to capture and tagging. The others, besides being of little or no commercial interest, were tagged in only very limited numbers.

Table I indicates the species from which recoveries have been received, the numbers tagged, and their ranges in length.

STARRY FLOUNDER (*Platichthys stellatus*)

Steady market acceptance, ruggedness, and association with species of greater commercial demand are conditions believed responsible for the comparatively good recovery of tags from starry flounder. The recoveries, most of

which were free for more than 300 days and some for as long as 700 days, indicate that starry flounders seldom move more than five miles. Twenty-one of the 23 recaptures useful for determining movement were within that range of dispersion. Two fish had made longer movements. One had moved 15 miles during 487 days at large. The other, a male, is notable in that it had travelled from Skidegate Inlet, Queen Charlotte Islands, to Rose Spit, approximately 60 miles to the north. It had been tagged for 2,037 days.

From tagged males which had remained free for approximately one year it was possible to estimate the average annual growth of male fish of different size. The increase in length of each male is plotted against its length at the time of tagging in Figure 1. The largest fish, 52 cm. long, had failed to grow

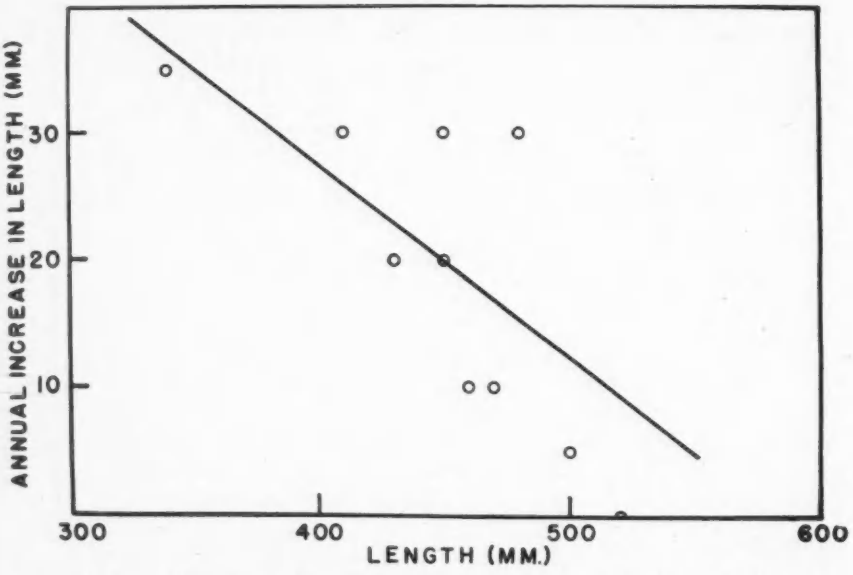

FIGURE 1. Annual increase in length of male starry flounders over length at time of tagging.

and the downward trend of the points for all males suggest that an "average" male would cease growth somewhere near 58 cm., the range being possibly from 50 cm. to nearly 60 cm. The largest male starry flounder actually measured during the investigation was 59 cm., and very few exceed 57 cm. A line fitted to the points for nine males 50 cm. or less in length indicates that, over that range, average annual growth of tagged males decreased 4 mm. for every 25 mm. increase in size of the fish. No data are available to estimate the rate of growth of females; however, female flatfishes commonly grow faster than do males.

SAND SOLE (*Psettichthys melanostictus*)

No particular reason can be given for the poor recapture of sand soles. Of the recoveries, one was taken in the area of tagging and another may have been. That the species may be migratory is shown by two females which were tagged off the Fraser River on November 22, 1944, and recovered 63 and 65 days later to the south-east in Boundary Bay approximately 25 miles away. Since the spawning of some populations of sand sole occurs in the late winter and early spring, this movement may have been a breeding migration. Another fish released in Imperial Eagle Channel, Barkley Sound, was recovered 632 days later in Mayne Bay, six miles away.

Some indication of the rate of growth can be gained from two fish. One, 30 cm. in length, increased 2 cm. in one year, while another 45 cm., increased a like amount in two years.

FLAT-HEAD SOLE (*Hippoglossoides elassodon*)

The very low recapture rate of flat-head soles is probably mainly due to differences between the fishing intensity on different grounds. Only one out of 166 tagged fish was recovered in areas rarely fished by trawlers; six out of 53 in areas moderately fished. No movement in excess of five miles is shown by any of the six returns for which usable data are available, two of which had been out for more than 800 days.

BLACKCOD (*Anoplopoma fimbria*)

Tagging of blackcod was carried out in Deep Water Bay. All recoveries were made, incidentally, by gear used principally for other species of fish. The relatively high recapture rate is accounted for by eleven tagged fish being taken by sunken dog-fish nets in the area of tagging within two weeks. Tags on blackcod of intermediate size greatly increase liability to capture in dogfish nets, as the tag catches in meshes which the fish otherwise pass through. Three fish were recaptured on the 140th day after tagging in Johnstone Strait, indicating movement into those waters during the early spring. The fact that these three individuals were together after almost five months is noteworthy. A further dispersion, towards Homfray Channel, was shown by one fish which had remained free for 224 days. During this period it had increased in length from 44 cm. to 48 cm., indicating a rather rapid growth.

GRAY COD (*Gadus macrocephalus*)

Because gray cod react badly to tagging, the number of fish tagged was small. Nevertheless two fish, one out for 43 days and the other for 99 days, were recovered. The fish out the shorter time, the only one for which adequate information is available, had travelled from Canoe Pass, Fraser River, to Boundary Bay. This movement is similar to that shown by some sand soles.

Oceanographic Data of the Western Canadian Arctic Region, 1935-37

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ABSTRACT

Hydrographic observations made incidental to summer patrol duties of the Royal Canadian Mounted Police vessel *St. Roche* showed a relatively warm (6.5-8°C.) saline (31.6‰) area in the Chukchi Sea, a cold (0.2-5.5 °C.) brackish (3-9‰) area from Point Barrow to Banks Island, and a cold (6.5-7.0 °C.) moderately saline (11.8-30.7‰) region from Banks Island through the channels to Coronation Gulf and Cambridge Bay.

During the time the Royal Canadian Mounted Police vessel *St. Roche* was on patrol duty in the Canadian Arctic from 1935 to 1937, Sergeant (now Inspector) H. A. Larsen, in charge, took a number of hydrographic observations with instruments supplied by the Pacific Biological Station, and also made some collections of biological material.

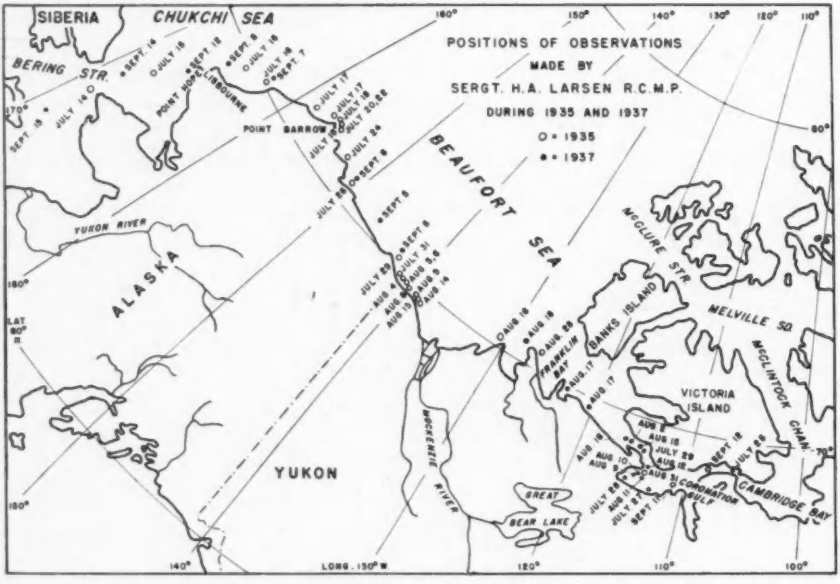

Figure 1. The route of the *St. Roche* and dates at each station.

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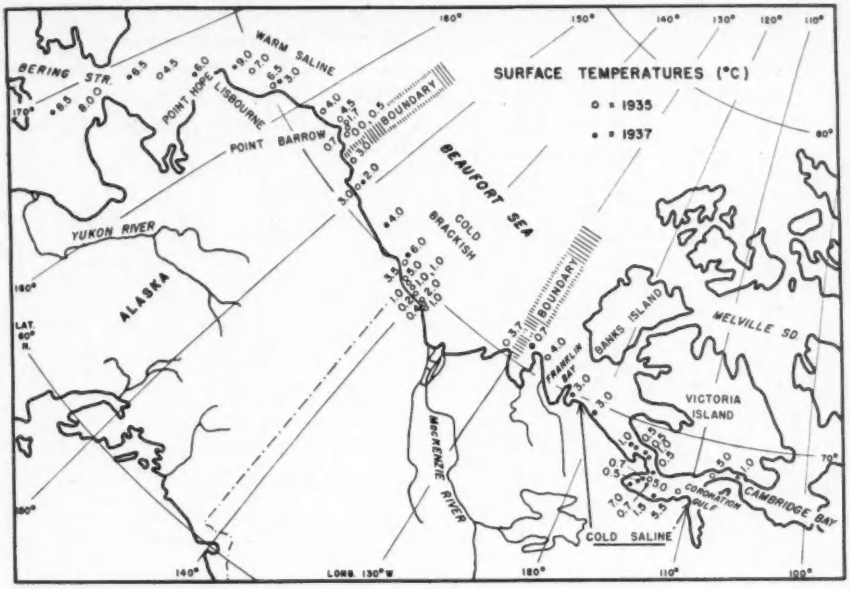

FIGURE 2. Surface sea-water temperatures at each station.

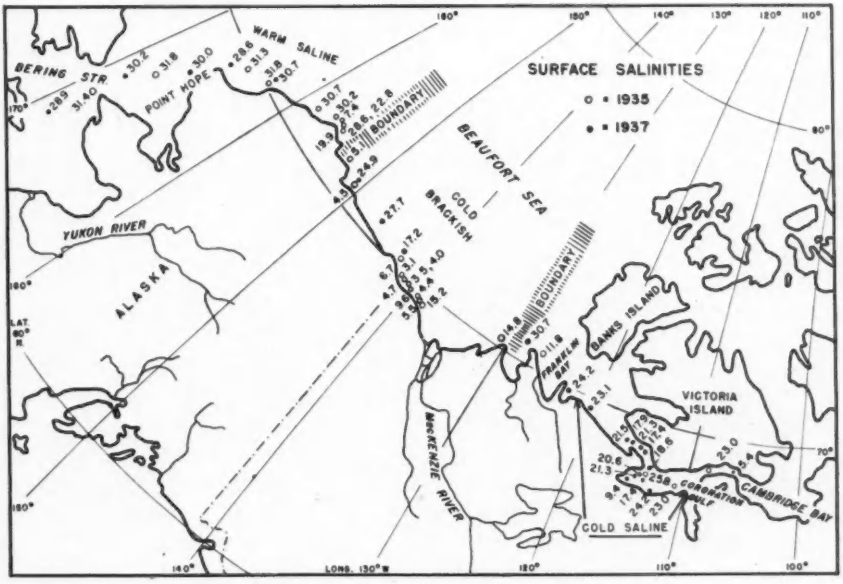

FIGURE 3. Surface sea-water salinities at each station.

The saga of Inspector Larsen's epic expedition has been fittingly described by G. J. Tranter in *Ploughing the Arctic* [1] and needs no comment here. The Fisheries Research Board of Canada is greatly indebted to the Royal Canadian Mounted Police and Inspector Larsen for these collections, which are the first of their kind in this region and contribute markedly to the knowledge of the biology and hydrography of Arctic waters.

TABLE I. Oceanographic data, 1935

Date	Latitude North	Longitude West	Specific gravity	Temperature	Calculated salinity
				°C.	S‰
July 14	65° 47′	168° 20′	1.0245	8.0	31.4
15	67° 28′	167° 17′	1.0252	4.5	31.8
16	69° 48′	164° 15′	1.0245	7.0	31.3
16	70° 10′	162° 38′	1.0249	6.5	31.8
17	71° 02′	158° 15′	1.0243	4.0	30.7
17	71° 25′	156° 20′	1.0240	4.5	30.2
18	71° 24′	155° 54′	1.0059	1.7	7.4
18	71° 20′	155° 40′	1.0160	0.7	19.9
20	71° 18′	155° 05′	1.0229	0.0	28.6
22	71° 19′	155° 05′	1.0183	0.5	22.8
24	70° 58′	152° 55′	1.0041	3.0	5.1
26	70° 38′	150° 50′	1.0037	3.0	4.5
29	70° 07′	142° 40′	1.0053	3.5	6.7
31	69° 45′	141° 20′	1.0025	5.0	3.1
Aug. 4	69° 45′	140° 45′	1.0037	1.0	4.7
5	69° 45′	140° 40′	1.0027	1.0	3.5
6	69° 45′	140° 40′	1.0032	1.0	4.0
8	69° 40′	140° 00′	1.0077	0.2	9.6
9	69° 40′	139° 00′	1.0036	2.0	4.4
14	69° 38′	138° 30′	1.0043	0.4	5.5
15	69° 32′	138° 50′	1.0122	1.0	15.2
16	70° 17′	130° 45′	1.0120	3.7	14.8
28	70° 28′	126° 20′	1.0095	4.0	11.8
31	68° 24′	113° 50′	1.0204	5.0	25.8
Sept. 11	68° 06′	110° 35′	1.0181	5.5	23.0
12	68° 50′	108° 20′	1.0181	5.0	23.0

The data and collections were deposited with the Pacific Biological Station in 1938. The biological material was sorted and submitted to specialists for study and reports have been published [2, 3, 4].

At first it was thought that the hydrographic observations were too few to define the condition, but recent advances in the knowledge of oceanographic principles have made it possible to intepret these to some extent, as a guide to further explorations.

Figure 1 shows the route of the *St. Roche* and dates at each position of observation. Figures 2 and 3 show the surface sea water temperature (°C.)

and salinity (S ‰, parts of salt per thousand parts by weight of sea water) at the positions. The data are cited in Tables I and II.

Temperatures were observed with a reversing thermometer [5]. Specific gravity was observed with standard sea water hydrometers and the salinity was computed from these two values by Knudsen's tables [6].

TABLE II. Oceanographic data, 1937.

Date	Time	Latitude North	Longitude West	Specific gravity	Temperature	Calculated salinity	Wind	Remarks
					°C.	S‰		
July 25	1200	68° 58′	106° 20′	1.0043	1.0	5.4	0	pack ice
27	1200	67° 46′	112° 40′	1.0195	1.5	24.2	NW7	drift ice
28	2100	67° 53′	115° 00′	1.0074	7.0	9.4	E2	cake ice
29	0800	68° 45′	114° 25′	1.0140	1.0	17.4	W4	drift ice
Aug. 2	1200	69° 03′	115° 37′	1.0143	0.5	17.9	NE4	floe ice
9	1900	68° 16′	114° 00′	1.0171	0.5	21.3	0	pack ice
10	0800	68° 18′	113° 55′	1.0166	0.7	20.6	NE	pack ice
11	0800	68° 20′	113° 45′	1.0148	0.7	17.4	0	fog pack ice
12	1200	68° 26′	113° 55′	1.0150	0.5	18.6	NE3	pack ice
15	0800	68° 51′	114° 41′	1.0172	0.5	21.3	NW2	cake ice
16	1600	69° 07′	116° 00′	1.0173	1.0	21.5	NW2	drift ice
17	0800	69° 39′	120° 28′	1.0185	3.0	23.1	SE2	no ice
17	1900	69° 53′	122° 50′	1.0193	3.0	24.2	SE3	drift ice
18	1200	70° 36′	128° 20′	1.0247	0.7	30.7	NE2	drift ice
Sept. 5	0800	70° 17′	142° 51′	1.0136	6.0	17.2	NW2	no ice
5	1900	70° 30′	146° 25′	1.0220	4.0	27.7	ENE4	no ice
6	1200	70° 56′	150° 57′	1.0200	2.0	24.9	ENE4	drift ice
7	2000	70° 17′	162° 50′	1.0245	3.0	30.7	NE8	no ice
8	1200	69° 20′	165° 45′	1.0221	9.0	28.6	NW2	no ice
12	1900	68° 16′	166° 30′	1.0236	6.0	30.0	NE8	no ice
14	0925	66° 42′	168° 52′	1.0238	6.5	30.2	N2	no ice
15	0800	64° 26′	168° 47′	1.0225	8.5	28.9	N2	no ice

From Bering Strait, through the Chukchi Sea to Point Barrow the sea water is relatively warm (6.5-8 °C.) and saline (31.6 ‰ S). As shown by the positions, the *St. Roche* kept a moderate (safe) distance offshore and so encountered water of intermediate salinity between the muddy coastal current, and Arctic ocean water.

Passing Point Barrow the *St. Roche* entered a second region of much fresher (brackish) (3-9 ‰ S) and colder (0.2-5.5°C.) water which extended to the mouth of the Mackenzie River and beyond to Banks Island. The freshness of this water is probably due to the river but the reason for its accumulation in this region is not apparent in the data. Normally it would be anticipated that the river outflow would veer to the right (eastward) and accumulate in the vicinity of Banks Island. This tendency may have been opposed by

northeasterly winds or could be due to the westward set of the Polar ice floes. During the return trip (1937) the higher temperatures and salinities consistent with water from the Chukchi Sea were in evidence, and probably represent the more normal conditions.

A marked increase of salinity was encountered in the vicinity of Franklin Bay which continued to the eastward limit of observations. The water in this region is cold and moderately saline (about two-thirds the salinity of ocean water) and probably originates in the mixing of the brackish surface water with the deep saline water of the Arctic Ocean. There is no indication in these data that the "freshwater influence" of the Mackenzie River continues eastward through the passages to Coronation Gulf and Cambridge Bay, as might have been expected by analogy with other areas.

The advance of Arctic research in recent years has indicated that this region is of considerable interest, and Inspector Larsen's observations are the only data indicating the oceanographic conditions along this western Arctic coastline. It is hoped that these may be extended in the near future to describe the oceanography of the Beaufort Sea and its eastern and western approaches.

REFERENCES

1. Tranter, G. J. Ploughing the Arctic. Toronto: Longmans Green, 1945.
2. Hart, J. F. L. Cumacea and Decapoda of the western Canadian Arctic Region, 1936-37. *Can. J. Res.*, D, **17**, 62-67, 1939.
3. Berkeley, E., and C. Berkeley. Polycheata from the western Canadian Arctic Region. *Can. J. Res.*, D, **22**, 1-5, 1944.
4. Fraser, C. McLean. Hydroids of the western Canadian Arctic Region, 1935-37. *Can. J. Res.*, D, **17**, 59-61, 1939.
5. Physics of the earth. V. Oceanography. *U. S. Nat. Res. Counc. Bull.*, No. 85. Washington, 1932.
6. Knudsen, M. Hydrographic tables. Copenhagen: G. E. C. Gad, 1901.